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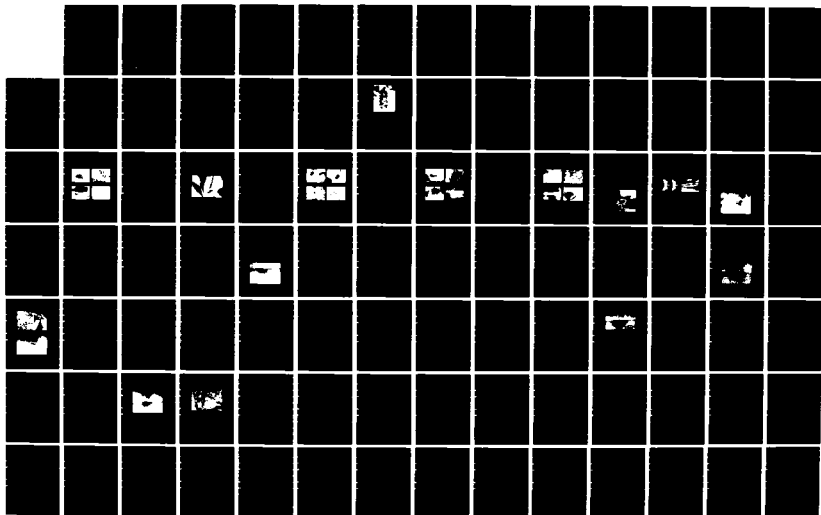
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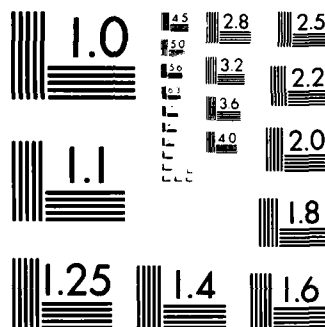
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) This report documents results of a Large-Scale Operations Management Test (LSOMT) of insects and plant pathogens for control of waterhyacinth in Louisiana during 1979-1981. The LSOMT consisted of five separate field studies as follows: a. <i>Cercospora</i> field application rate study.		

(Continued)

20. ABSTRACT (Continued).

- b. *Neochetina*, *Sameodes*, and a spring application of the original *Cercospora* formulation.
- c. *Neochetina* and a spring application of a modified *Cercospora* formulation.
- d. *Neochetina* and *Sameodes*.
- e. Establishment, dispersal, and distribution of *Sameodes*.

The purpose of the studies was to determine which agents or agent combinations provided the greatest degree of control, and to determine the level of waterhyacinth control that each agent or agent combination could provide.

During the study, the population of waterhyacinth in Louisiana declined from 1.2 million acres to 300,000 acres. Results from the field studies implicated *Neochetina eichhorniae* Warner (mottled waterhyacinth weevil) as the principal factor responsible for the observed decline in waterhyacinth. The waterhyacinth population at the site used for the *Cercospora* field application rate study decreased by approximately 90 percent from April to September 1980, due principally to a dense population of *Neochetina*. The waterhyacinth biomass at all other test areas decreased significantly during 1980 or 1981, but there were no reductions in percent cover. Continued expansion of *Neochetina* populations in study areas during 1981 suggested that greater impacts on the waterhyacinth population might occur in 1982.

Cercospora rodmanii Conway (waterhyacinth leaf spot fungus) became successfully established at only one of three sites where it was applied. Disease symptoms produced by *Cercospora* continued to increase in abundance throughout 1981 at one site, and the fungus appeared to be significantly stressing the plant population. Failure of *Cercospora* to become established at two sites was attributed to severe, rapid impacts of a dense *Neochetina* population on the waterhyacinth population at one site, and a lack of virulence of *Cercospora* in the formulation applied at the other site.

Sameodes albiguttalis Warren (Argentine waterhyacinth moth) became established on waterhyacinth in one release area in 1979 and rapidly dispersed during 1980 and 1981. By October 1981, the distribution of *Sameodes* encompassed a 2883-km² area, including all or portions of nine parishes. However, population development sufficient to produce noticeable impacts on waterhyacinth populations had occurred in only a few areas. Although too early to predict the level of control that could be effected by *Sameodes*, its effectiveness may be limited by significant mortality of overwintering populations. Large *Sameodes* populations were not observed until late summer and fall.

Biological agents, especially *Neochetina eichhorniae*, were demonstrated to provide an effective, long-term method for controlling waterhyacinth. The ultimate degree of control remained to be determined, but the insects and plant pathogens are not expected to eradicate waterhyacinth or provide the desired level of control in all areas.

PREFACE

This research was sponsored by the US Army Engineer District, New Orleans (LMN), and the Office, Chief of Engineers (OCE), US Army, Washington, DC, through the Aquatic Plant Control Research Program (APCRP) at the US Army Engineer Waterways Experiment Station (WES). The OCE Technical Monitors during the study and report preparation were Messrs. H. Roger Hamilton, Dwight Quarles, and E. Carl Brown.

This report (Volume I) describes the results of a series of studies conducted as part of a Large-Scale Operations Management Test (LSOMT) of insects and pathogens for the control of waterhyacinth in Louisiana. Specifically, the report documents results obtained from 1979 through 1981, at which time LMN funding was terminated due to fiscal constraints. A second report (Volume II) will be produced that documents results and conclusions obtained during 1982 and 1983. Findings of this research will be applicable to all Corps Districts in which waterhyacinth occurs at problem levels by identifying effective biocontrol agents and combinations of agents, demonstrating their level of effectiveness, and describing methods for monitoring biocontrol agent populations and their impacts on waterhyacinth populations.

This report was prepared by Dr. Dana R. Sanders, Sr., and Mr. Edwin A. Theriot, both of the Wetland and Terrestrial Habitat Group (WTHG), Environmental Resources Division (ERD), Environmental Laboratory (EL), WES, and Dr. Patricia Perfetti, University of Tennessee-Chattanooga, Chattanooga, Tennessee. The field research and data analyses were performed by Dr. Alfred F. Cofrancesco and Messrs. R. Michael Stewart and Samuel O. Shirley, all of the WTHG. Mr. Russell F. Theriot, WTHG, served as Principal Investigator of this study.

Special field assistance was provided by Mr. James Manning, Louisiana Department of Wildlife and Fisheries, Baton Rouge, La. Abbott Laboratories, Inc., Chicago, Ill., provided the *Cercospora* formulations evaluated in this study. Dr. Ted Center and Mr. Wiley Durden, both of the US Department of Agriculture Aquatic Plant Management Laboratory, Fort Lauderdale, Fla., provided colonies of *Sameodes* used in some releases, and participated in field surveys for *Sameodes*.

The research was conducted under the direct supervision of Dr. Hanley K. Smith, Chief, WTHG; and under the general supervision of Dr. Conrad J. Kirby, Jr., Chief, ERD; and Dr. John Harrison, Chief, EL. Mr. J. Lewis Decell was Program Manager for the APCRP.

Commanders and Directors of the WES during the study and report preparation were COL John L. Cannon, CE, COL Nelson P. Conover, CE, and COL Tilford C. Creel, CE. Technical Director was Mr. Fred R. Brown.

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CONVERSION FACTORS, U. S. CUSTOMARY TO
METRIC (SI) UNITS OF MEASUREMENT

<u>Multiply</u>	<u>By</u>	<u>To obtain</u>
acres	0.4047	hectares
Fahrenheit degrees	5/9	Celsius degrees or
feet	30.48	centimetres
gallons	3.785	litres
		Kelvins*
inches	2.54	centimetres
miles per hour	1.60347	kilometres
miles (U. S. statute)	1.609347	kilometres
pints (U. S. liquid)	0.4731765	cubic decimetres
pounds (force) per square inch	6894.757	pascals
pounds (mass)	0.454	kilograms

* To obtain Celsius (C) temperature readings from Fahrenheit (F) readings, use the following formula: $C = (5/9)(F - 32)$. To obtain Kelvins (K) readings, use $K = (5/9)(F - 32) + 273.15$.

LARGE-SCALE OPERATIONS MANAGEMENT TEST (LSOMT) OF INSECTS AND
PATHOGENS FOR CONTROL OF WATERHYACINTH IN LOUISIANA

Volume I: Results for 1979-1981

PART I: INTRODUCTION

Background

1. Waterhyacinth [*Eichhornia crassipes* Mart. (Solms)], an aggressive floating aquatic plant species, was introduced into this country at the 1884 Cotton States Exposition, New Orleans, La. Waterhyacinth has flourished in an environment where the plant and animal species that limit its growth in its native range are absent. Waterhyacinth occurred in nearly all southern Louisiana waterways by 1900 (Raynes 1964), and now threatens 4.7 million of Louisiana's 6.4 million acres* of freshwater habitat. It has spread across the southeastern United States and presently occurs throughout the southern states from Texas to South Carolina, and in California (Figure 1).

2. Waterhyacinth adversely impacts man's use of waterways in several ways when unchecked. Massive populations of waterhyacinth impede navigation, restrict all types of water-oriented recreational activities (i.e. boating, swimming, fishing), reduce water movement through flood control and irrigation systems, increase water loss through evapotranspiration, and threaten the structural integrity of bridges. Waterhyacinth mats are detrimental to fishery resources by shading out submersed aquatic species that typically serve as oxygenators of the water, food for juvenile fish, and shelter for commercial and game fish. These mats are also detrimental by affording protected habitat for reproduction of species of mosquitos that vector several human diseases. When such impacts occur, management efforts must be undertaken to reduce the waterhyacinth populations to a nonproblem level.

3. The 1899 River and Harbor Act authorized the US Army Corps of Engineers (CE) to conduct waterhyacinth control activities. Earliest control

* A table of factors for converting U. S. customary units of measurement to metric (SI) units is presented on page 6.



Figure 1. Distribution of waterhyacinth in the United States

efforts consisted of barriers to prevent downstream movement of waterhyacinth mats, and large mechanical systems designed to physically remove the mats from navigable waterways. Very little was initially known about the growth habit or life cycle of waterhyacinth; thus, control efforts were carried out only during the growing season because the plant was thought to be dormant during winter. Management operations began with a slow, cumbersome mechanical sugar mill crusher placed on the bow of a steamboat and fed by a pick-up conveyor that delivered plant material from the water surface to the crusher. The crushed refuse was returned to the water. Mechanical crushers, while effective in destroying the vegetation, could not keep pace with the rapid proliferation of waterhyacinth, particularly since their use was confined to a seasonal schedule (Jernigan, Tabita, and Wunderlich 1964).

4. Although mechanical control historically preceded chemical control, the first large-scale control of waterhyacinth in Louisiana was achieved chemically. Sodium arsenite, which provided total control of treated

Reproduction

41. Sexual reproduction in *C. rodmanii* has not been observed and reproduction occurs only by asexual spores (conidia). The generation time is 3 to 6 weeks. Maximum sporulation occurs at 20° to 30°C, and sporulation is inhibited at 10°C.

Infection

42. Infection by *C. rodmanii* occurs when conidia or mycelia are transferred from a diseased plant to an uninfected plant by wind or direct contact. Rate of infection is determined by the number of spores that land on the leaves. When a spore germinates, a threadlike hyphum grows across the leaf surface until it reaches a stomatal opening. The hyphum grows into the stomata and penetrates the substomatal chamber. Hyphal growth accelerates and a mycelial network is produced that penetrates the inner leaf tissues. The mycelium destroys leaf tissues by extracellular digestion. Plant necrosis is accelerated by the endotoxin, cercosporin.

Symptoms on waterhyacinth

43. The leaf spot disease caused by *C. rodmanii* is characterized by the following symptoms:

- a. Punctate leaf spots form near the apex of the leaf blade. Usually, chlorosis of the leaf blade is also visible.
- b. As fungal proliferation continues, the leaf spots coalesce and the leaf apex becomes necrotic.
- c. Later, the entire leaf blade and petiole become necrotic.

Host specificity

44. Conway and Freeman (1977) tested *C. rodmanii* under greenhouse conditions on 85 plant species, representing 58 species from 22 plant families. Only squash, cucumber, and spinach were infected, and damage on these species was restricted to older, dying leaves. Repeated tests on squash and cucumber produced no evidence of disease symptoms. *Cercospora rodmanii* produced disease symptoms on only two varieties of lettuce in field tests. However, the test plants were also infected by an *Alternaria* species, and infection by *C. rodmanii* was considered to be secondary. *Cercospora rodmanii* is not infectious on other species of aquatic plants (Conway and Freeman 1977).

45. Since *C. rodmanii* will not grow at 37°C, it poses no threat to man. Mosquitofish (*Gambusia affinis* Baird and Girard) is not affected by *C. rodmanii* (Freeman et al. 1981). *Cercospora rodmanii* is also nontoxic to mice,

PART III: THE BIOCONTROL AGENTS

38. The taxonomy and characteristics of each biocontrol agent, life cycles, and typical impacts on waterhyacinth are described in this part.

Cercospora rodmanii Conway

Taxonomy

39. A leaf-spot fungus associated with a natural decline in a waterhyacinth population at Rodman Reservoir, Florida, in 1971 was found to be a previously undescribed species of *Cercospora* (Conway, Freeman, and Charudattan 1974). Conway (1976) described the species and named it *Cercospora rodmanii* Conway sp. nov. (Form Class: Fungi Imperfecti).

Description

40. Conway (1976) described *Cercospora rodmanii* as follows:

Leaf spots black, punctate to circular (1-3 mm diam), leaf and petiole chlorotic, tip of leaf necrotic, conidiophores amphigenous, 3-12 in each fascicle, brown sympodial, arising from a well developed stroma, emerging through the stoma, 84 - (145 × 4 - (4.5) - 5 μm; conidia hyaline, truncate at base, acicular, multiseptate, 66 - (172) - 374 × 3 - (4) - 5 μm.

A pycnidial state was described as follows:

Asteromella pycnidia dark brown, ostiolate, globose, 80-95 × 80-110 μm, substomal, later erumpent, ostiole 30-40 × 25-30 μm; conidia hyaline, bacilliform 2-3.5 × 1-1.5 μm.

Cercospora rodmanii is very similar to another species, *Cercospora piaropi* Tharp, first isolated from waterhyacinth in Texas in 1917. *Cercospora rodmannii* differs from *C. piaropi* in the following characteristics (Conway 1976):

<u><i>C. rodmanii</i></u>	<u><i>C. piaropi</i></u>
Punctate to circular leaf spots	Discrete spots
Tip dieback	No tip dieback
Amphigenous conidiophores	Epiphyllous conidiophores
Nine or fewer conidiophores per fascicle	Three to twelve conidiophores per fascicle
Conidiophore length 66-374 mm	Conidiophore length 55-200 mm
Conidial base truncate	Conidial base obconic
Conidial size 66-374 mm	Conidial size 25-220 mm
Well-developed stroma	Stroma lacking or only a few cells

a variety of arthropods and pathogens on waterhyacinth in Louisiana, but they produced only minimal impact on plant growth. Naturally occurring arthropods observed were *Arzama*, *Orthogalumna*, and several species of grasshoppers. Five fungal pathogen genera other than *Cercospora* were observed: *Fusarium*, *Helminthosporium*, *Nigrospora*, *Alternaria*, and *Acremonium*. Six bacterial isolates were identified: *Pseudomonas*, *Xanthomonas*, *Achromobacter*, *Proteus*, *Erwinia*, and *Aerobacter*.

Waterhyacinth growth rates and plant size are also influenced by nutrient availability. The critical limiting concentration of phosphorus is 0.10 mg/l and of nitrate is 0.50 to 1.0 mg/l (Haller, Knipling, and West 1970; Boyd 1976).

Biotic factors

35. Waterhyacinth is significantly impacted by many biological interactions, including intraspecific and interspecific plant competition, parasites, and predators. Seasonal increases in plant density and size are influenced by the degree of intraspecific competition (Center and Spencer 1981). Center and Spencer contended that the tendency of waterhyacinth to produce a high canopy in crowded conditions reflects a rapid adjustment in leaf size and shape, which results in a redistribution of biomass and a leaf form optimal for crowded conditions. When competition is intense, petiole elongation enables the leaves to grow above neighboring plants. Equitant leaves are maintained at an average compensation point where $P \geq R^*$ until environmental conditions intervene. In contrast, an increase in plant density is triggered by ramet production as the canopy decreases in height and the population becomes less crowded in the declining phase (Center and Spencer 1981).

36. The unparalleled ability of waterhyacinth to reproduce vegetatively enables the plant to rapidly dominate available space and preclude competition from other species (Center and Spencer 1981). Competitive studies of waterhyacinth and waterlettuce (*Pistia stratiotes* L.) revealed waterhyacinth to have the clear advantage over waterlettuce at pH 4, 7, and 9 (Tag El Seed 1978). He concluded that waterhyacinth is successful in eliminating waterlettuce because the larger leaves of waterhyacinth give it a competitive advantage in establishing a canopy over the water surface.

37. Prior to the 1970's, there were virtually no parasites or herbivores in the United States that significantly impacted waterhyacinth populations. However, several species were found to exert a low degree of stress, including: (a) *Arzama densa* Walker, a native moth whose larvae feed preferentially on pickerelweed (*Pontederia cordata* L.), but also on waterhyacinth to some extent; (b) *Orthogalumna terebrantis* Wallwork, a mite apparently adventively introduced from South America; and (c) certain plant pathogens (*Cercospora*) from Florida (DeLoach and Cordo 1978). Foret, Barry, and Theriot (1980) found

* Photosynthesis is greater than or equal to respiration.

Sexual reproductive cycle

31. Abundant flowering occurs on waterhyacinth plants of all sizes except the Stage III plants. Although the flower appears to be well adapted for entomophily, pollination by insects has rarely been observed and self-pollination is the general rule (Penfound and Earle 1948).

32. Gowanloch (1944) estimated an average annual production of 500 seeds per plant. Other seed production estimates range from 1 to 45 million seeds per acre in a growing season. This number, when multiplied by the 15 to 20 years that a seed may remain viable, emphasizes the enormous reproductive potential of waterhyacinth. Although only a small percentage (~5 percent) of the seeds germinate, species survival is ensured even when the total standing crop is destroyed. Thus, waterhyacinth has never been eradicated from any region to which it has been introduced (Penfound and Earle 1948, Center and Spencer 1981).

Influence of Environmental Factors on the Growth Cycle

33. The above-described growth cycle of waterhyacinth is influenced by a complex system of interacting abiotic and biotic factors. A brief review of factors known to influence the waterhyacinth growth cycle follows. Although the factors are treated independently, they act collectively and simultaneously, influencing each other and in turn being influenced.

Abiotic factors

34. Waterhyacinth phenology and growth are influenced by length of growing season, annual temperature and solar radiation patterns, and nutrient availability. Penfound and Earle (1948) hypothesized that transition in leaf type was triggered by changes in light intensity. They found that bulbous-petioled leaves form only when average light intensity exceeds 500 ft-candles, and that equitant leaves form at intensities ranging from 130 to 500 ft-candles. Center and Spencer (1981) suggested that ramet production is contingent on light penetration beyond the uppermost leaves and ceases under a full canopy. After maximum biomass production occurs at the peak of the growing season, standing crop values track climatic conditions in a "steady-state" situation. When reduced temperatures and photoperiod occur in the fall, leaf and individual plant size decline as well as overall canopy height.

described. Ten individual plants were reported by Penfound and Earle (1948) to produce a mat of 655,360 plants per acre during one growing season (15 March to 15 November) in the New Orleans area. Center and Spencer (1981) estimated an absolute rate of increase between 10 and 20 g/m² (dry weight) per day for waterhyacinth in a eutrophic lake in north-central Florida. Thus, waterhyacinth is one of the most productive plant species (Westlake 1963).

29. Phenotypic summer. The summer phase begins when a mat created by the interweaving of stolons of daughter plants has resulted in a closed canopy. Under such crowded conditions and provided that adequate nutrients are available, ramet production is reduced and the Stage I plants convert to a tall, equitant leaf morphotype with elongate petioles. As this process occurs, flowering is initiated and the intermediate flowering form, which has both types of petioles, is referred to as the Stage II morphotype. The plants become increasingly robust, sometimes reaching a height of 1 m or more while maintaining an average complement of six to eight leaves. Intraspecific competition intensifies as space becomes limited, and the smaller plants are out-competed by the taller, faster growing ones, thereby resulting in a natural thinning of the population. Flowering seldom occurs on larger plants, which are referred to as the Stage III morphotype. Petioles are structurally important in this phase of intense competition for light because they function in displaying the leaves of taller plants above neighboring plants and are positioned almost vertically at a 75- to 90-deg angle from the water surface (Penfound and Earle 1948). The greatest accumulation of biomass occurs during this period (mid-March through mid-June in Florida). However, net production decreases by June when most photosynthate becomes required for respiration (Center and Spencer 1981).

30. Phenotypic fall. The summer phase passes into a declining phase with the onset of cooler temperatures and shorter photoperiods in fall. Canopy thinning occurs during September and October in Louisiana when the rate of leaf production decreases. Although Center and Spencer (1981) observed ramet production in Florida in October and November and predominantly small plants by December, ramet production seldom occurs during this period in Louisiana. Instead, frosts occurring during late October and November in Louisiana progressively kill the tall plants. Necrotic leaves persist for a time, but they eventually drop off, leaving only the submersed rhizome and roots.

25. Phenotypic winter. Waterhyacinth is a herbaceous plant that undergoes considerable dieback of exposed parts due to occasional frosts that occur in subtropical climates during December and January. Frost damage results in substantial leaf dieback. However, foliar insulation and the usual position of the rhizome an inch or so below the water surface prevent most rhizome apices from being destroyed. Only extreme or repeated cold periods result in rhizome destruction (Vietmeyer 1975). Penfound and Earle (1948) estimated that 30 to 90 percent of the waterhyacinth plant cover was eliminated from small, exposed bodies of water in the New Orleans area during an extended cold period in January 1940 when freezing temperatures were experienced on 12 successive nights.

26. Phenotypic spring. With the advent of warmer temperatures (February-March), the viable rhizomes grow monopodially, producing their first whorled complement of six to eight new leaves. These leaves (20 cm long and 5 to 15 cm wide) consist of leathery, orbicular blades and greatly inflated, bulbous petioles. The petioles serve as floats and the leaves are oriented in a nearly horizontal position at an angle varying from 15 to 45 deg above the water surface (Penfound and Earle 1948). Plants of this type are referred to as the Stage I morphotype.

27. Colonization of an open body of water by ramet (Center 1981b) production begins soon after development of a leaf complement. Ramets, which are vegetatively propagated daughter plants, arise on stolons produced by sympodial rhizome branching. Ramets act as colonizers when the brittle stolons by which they are connected to the parent plant are broken (Bock 1969). Center and Spencer (1981) reported peak densities as high as 180 plants per square metre in April in a eutrophic north-central Florida lake. Ramet production proceeds until either a dense, high, monolayered canopy forms or environmental conditions intervene. As colonization takes place, ramet production commonly ceases in the center of a mat, but continues at the fringe. Fringe plants are usually the Stage I morphotype.

28. The colonization phase, which occurs during February to May in Louisiana, is characterized by high net primary production, when $P_g/R > 1^*$ (Center and Spencer 1981). Various productivity estimates have been

* $\frac{\text{Gross Productivity}}{\text{Respiration}}$ is greater than 1.



Figure 3. Waterhyacinth inflorescence

from a spathe. The perianth is tubular at the base and six lobed, the upper lobe having a distinctively deeper blue-violet blotch with a yellow, diamond-like center (Figure 3).

23. Waterhyacinth reproduces both asexually and sexually. Asexual reproduction (vegetative propagation and fragmentation) occurs more commonly and is more important than sexual reproduction. Bock (1969) reported that reproduction occurred only by vegetative means in California. Sexual reproduction occurs in Louisiana, but the reproductive cycle is not completed in one growing season (Penfound and Earle 1948). Thus, sexual reproduction occurs more slowly than vegetative reproduction. Waterhyacinth is capable of asexually doubling its plant numbers in approximately 2 weeks (Penfound and Earle 1948).

Vegetative growth cycle

24. The morphology of waterhyacinth varies seasonally and with the amount of crowding. Phenological changes associated with the annual pattern of vegetative growth are presented in the following paragraphs.

PART II: THE PROBLEM PLANT - WATERHYACINTH

Taxonomy and Range

21. Waterhyacinth was first reported in the botanical literature in 1824 when Karl von Martius described it from Brazil as *Pontederia crassipes* Mart. (synonym *Piaropus crassipes*). Since its distribution was apparently limited and it was not described several centuries earlier, waterhyacinth was considered native to Brazil. Its natural range was restricted to tropical South America, and perhaps parts of Central America and the larger Caribbean islands (Pieterse 1978). Its current adventive range extends throughout virtually all tropical and subtropical areas (Vietmeyer 1975). The species was reassigned to the genus *Eichhornia* by Solms-Laubach in 1883 (Pieterse 1978). Waterhyacinth is a member of the Pontederiaceae (pickerelweed family). Although seven genera are recognized worldwide (Pieterse 1978), Godfrey and Wooten (1979) list only two related genera (*Pontederia* and *Heteranthera*) that commonly occur in the southeastern United States. All three genera (including *Eichhornia*) found in the southeastern United States are perennials of freshwater habitats and spread vegetatively by horizontal stem growth from creeping or floating rhizomes. Waterhyacinth is best adapted to tropical riverine systems because of its free-floating growth habit. However, waterhyacinth may become anchored in the hydrosol during low water periods.

Phenology and Life Cycle

Morphology and reproduction

22. Waterhyacinth is readily identified by its distinctive vegetative and reproductive morphology (Figure 3). In the vegetative condition, plants consist of radiating clusters of thick aerial leaves with suborbicular to broadly elliptic leaf blades. Rhizomes and roots are submersed. The black, hairlike roots are suspended in the water column in featherlike tufts. Juvenile waterhyacinths are more buoyant than mature plants because their modified petioles have a specific gravity considerably lower (0.14) than those of the other plant parts (0.74 to 0.82) (Penfound and Earle 1948). The reproductive plant has a short, erect inflorescence of blue zygomorphic flowers arising

to be evaluated were *Cercospora rodmanii*, *Neochetina eichhorniae*, and *Sameodes albiguttalis*.*

17. Objectives of the LSOMT were:

- a. Determine the level of waterhyacinth control provided by various biocontrol agents, used both alone and in combinations.
- b. Determine the most effective combination of biocontrol agents for waterhyacinth control in Louisiana.
- c. Develop the framework of an operational system for routinely using biological agents for waterhyacinth control.

Purpose of Report

18. The purpose of this report is to present the LSOMT results. Since the LSOMT consists of a series of component tests relating to each biocontrol agent and various combinations of agents, the report will describe each component test separately.

Scope and Content of Report

19. This report focuses on a series of tests that address the general LSOMT objectives. It also includes a summary of previous studies leading to the LSOMT, as well as basic information on the biology of waterhyacinth and biocontrol agents.

20. Part II describes the waterhyacinth life cycle and phenology. The biocontrol agents and their life cycles are reviewed in Part III. Part IV summarizes preliminary tests of biocontrol agent efficacy. The series of tests comprising the LSOMT are described in Part V. Questions directly relevant to the efficient large-scale application and management of the organisms are also discussed in Part V. Part VI is a general discussion of overall results and Part VII presents conclusions.

* Henceforth, except in Part III of this report, these species will be referred to as *Cercospora*, *Neochetina*, and *Sameodes*.

biological control agents were approved for release. These included two species of waterhyacinth weevils (*Neochetina bruchi* Hustache and *N. eichhorniae* Warner) and the waterhyacinth moth (*Sameodes albiguttalis* Warren).

14. A new species of leaf-spot fungus (*Cercospora rodmanii* Conway) was found on waterhyacinth in Florida in 1970 (Conway 1976). Subsequent research led to development of a potentially commercial formulation of the fungus by Abbott Laboratories, Inc., Chicago, Ill. The CE conducted small-scale field trials in 1977 of combinations of all of these species except *Sameodes albiguttalis*. The candidate organisms produced some detrimental effects on waterhyacinth, but their potential was only partially realized (Addor 1977). However, there was evidence that combinations of these biocontrol agents could significantly impact waterhyacinth. Sanders et al. (1979) suggested using multiple agents to produce a synergistic effect, thereby effecting a greater degree of waterhyacinth control than provided by individual agents. A decision was made to proceed with an LSOMT. If effective control of waterhyacinth by biological agents could be demonstrated in the LSOMT, biological control would be a viable, long-term option for the management of waterhyacinth.

Definition and Objectives of the LSOMT

15. An LSOMT is a field test of proposed methods for the control of aquatic plants, conducted on selected large areas at a scale, and in a manner representative of, a full-scale field operations activity (Sanders et al. 1979). Its purpose is the transfer of basic, experimental research results to an applied, field-operational context. It bridges the gap between pure science and operations management by providing a test design and monitoring schedule integral to scientific research, but at a scale, and with minimal experimental controls, typical of a field operations activity.

16. This LSOMT was designed to determine whether or not the use of multiple biological control agents, demonstrated to be effective in laboratory and controlled small-scale field studies, provided effective and environmentally acceptable control of waterhyacinth at a field operations scale. Agents

9. Economic losses due to waterhyacinth have decreased in Louisiana in recent years as a direct result of the Federal- and State-sponsored management program. The immediate economic benefit of maintaining open waters in Louisiana for 1981 was calculated by the LDWF to be \$651,522,583 (LDWF 1981). This figure was calculated on the basis of acres maintained for sport fishing, without consideration of other benefits (e.g. flood control, irrigation, hunting, trapping, boating, navigation, and commercial fishing). The LDWF estimated that approximately 75 percent of the 4.2 million population of Louisiana received either direct or indirect benefits from the aquatic weed control program in 1981.

Rationale for Biological Control

10. The use of biological control is economically advantageous since there are few continuing operational costs beyond the initial capital costs of discovering, evaluating, and releasing the agents (Grabau 1977). A considerable long-term savings in the cost of other control methods might also occur if the overall infested acreage could be significantly reduced by biocontrol agents.

11. Biological control of aquatic plants was successfully demonstrated by the use of insects to control alligatorweed [*Alternanthera philoxeroides* (Mart.) Griseb.]. In view of this success, biological control appeared promising as a possible long-term management option for waterhyacinth control.

12. Although a serious plant pest in the United States, waterhyacinth is not a problem in Argentina, usually extending just a few yards from shore and only occasionally spreading sufficiently to block small waterways (DeLoach and Cordo 1976a). This is true even though many environmental parameters in both the United States and Argentina waters are similar. It was postulated that organisms using waterhyacinth as a food source in Argentina were responsible for controlling its rate of population development. This hypothesis stimulated CE-funded field exploration in the early 1960's by the US Department of Agriculture (USDA) throughout South America for candidate species that might be imported into the United States for waterhyacinth control (Center 1981a).

13. After more than 10 years of research, including screening and host-specificity studies in Argentina and in quarantine in the United States, three

infestation of 1,725,000 acres in 1975 (Figure 2). Consequently, alternative long-term management methods were sought.

8. Waterhyacinth control efforts accelerated in 1965 when Congress authorized establishment of the Aquatic Plant Control Program (APCP) under Section 302, Public Law 89-298 (79 USC 1092), River and Harbor Act of 1965, which included provisions for the Aquatic Plant Control Research Program (APCRP). This and other subsequent legislation provided increased funding for operational management, as well as research and development of alternative management approaches (Hamilton 1978). Research efforts on biological control of waterhyacinth, including the Large-Scale Operations Management Test (LSOMT) with insects and pathogens conducted by the US Army Engineer Waterways Experiment Station (WES) with funding provided by the US Army Engineer District, New Orleans (LMN), were a direct result of this legislation. Biological control was studied as an alternative to the chemical approach because biocontrol agents, once successfully established, are self-perpetuating and provide a low-cost, long-term remedy.

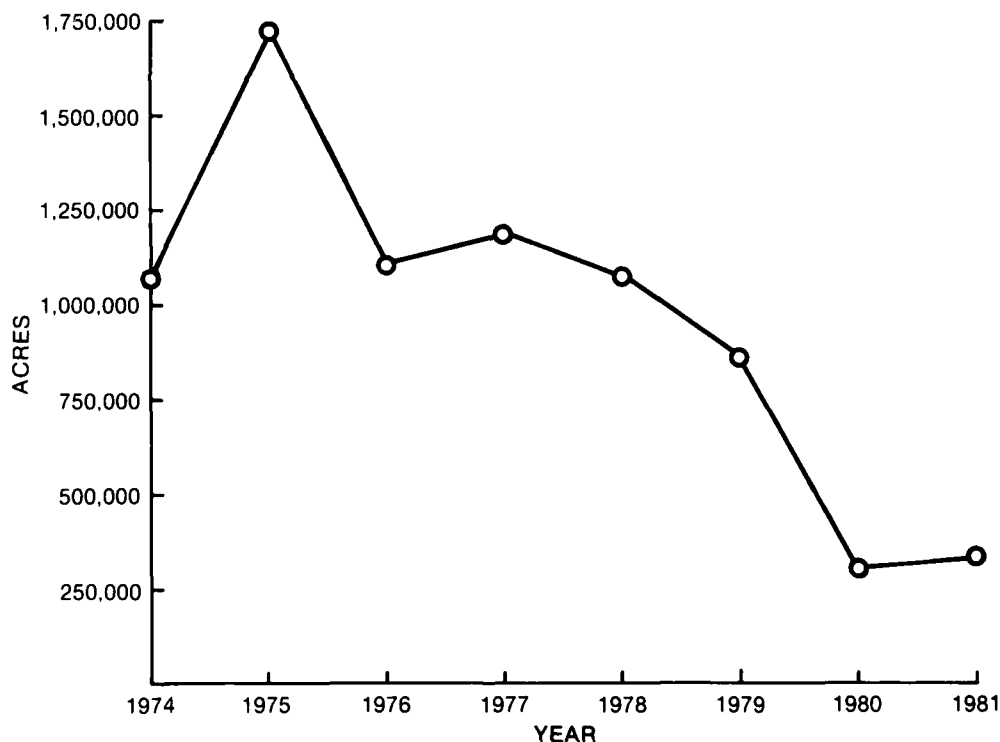


Figure 2. Total acreage of waterhyacinth in Louisiana during 1974-1981. Data provided by the Louisiana Department of Wildlife and Fisheries

waterhyacinth populations in 21 days, was adopted in 1902 before its environmental effects were known. With stringent safety precautions, sodium arsenite was utilized until 1937 with few serious accidents. Nevertheless, the herbicide killed cattle, damaged vegetation, and poisoned spray crews, causing at least one death. The use of sodium arsenite was discontinued in 1937 (Wunderlich 1962, 1964).

5. The first mechanical crusher served as the prototype for KENNY, a vastly improved, self-propelled crusher that was capable of destroying 210 acres of surface vegetation each month. KENNY was used on a continuous basis from 1937 to 1951 since by this time it was evident that more than seasonal control was necessary. This crusher is credited with the successful opening of many waterhyacinth-entrapped streams in southern Louisiana (Jernigan, Tabita, and Wunderlich 1964; Wunderlich 1962, 1964). In addition, conveyors and small mechanical harvesters were developed to operate in the shallower waters of feeder streams. The most versatile and effective mechanical harvester was the Louisiana model of the saw boat, which had gin saws mounted on the bow and side.

6. Efforts to keep navigable waterways open during the 1940's with mechanical systems were replaced in the 1950's by routine chemical spraying of phenoxy herbicides. The use of 2,4-D (2,4-dichlorophenoxyacetic acid) proved to be so effective that it has been the predominant method for the management of waterhyacinth since 1950. However, while extremely effective in providing short-term control in navigable waters, herbicide use is limited in backwater areas. These backwater areas serve as breeding or nursery grounds for waterhyacinth, providing a continuous supply of plants to the connecting navigable waterways during periods of high water. Since 2,4-D only provides short-term control, spraying must be repeated on a seasonal basis. Thus, the chemical control program is costly and does not provide a long-term solution to the problem.

7. As authorized by Public Law 85-500, the waterhyacinth management effort became a joint venture of the Louisiana Department of Wildlife and Fisheries (LDWF) and the CE in 1959. Although chemical control had been remarkably effective in maintaining nearly 3000 miles of open waterways in Louisiana since 1959, a major flood in 1973 disseminated waterhyacinth from backwater nursery areas so that the waterhyacinth population reached a peak

rats, rabbits, pheasants, and mallard ducks at exposure levels 100 to 1000 times higher than recommended application rates.*

Sameodes albiguttalis Warren

Taxonomy

46. *Sameodes albiguttalis*, the Argentine waterhyacinth moth, was described by Warren in 1889 as *Epichronistis albiguttalis* from three adult females collected in 1874 from Brazil. Hampson (1899) placed *Epichronistis albiguttalis* in the genus *Pyrausta*. The species was later named *Sameodes snellen*, which Hampson (1918) considered to be synonymous with *Epipagis*. More recent literature refers to *albiguttalis* as either *Epipagis* or *Sameodes*, although it apparently belongs to an unnamed genus and is neither a species of *Sameodes* nor *Epipagis*. Monroe recommended that it be provisionally placed in the genus *Sameodes* (DeLoach and Cordo 1978). *Sameodes* is a member of the Pyralidae family, a large and diverse group of relatively small, undistinguished moths with more than 1100 species in North America. *Sameodes* occurs most often in fringes of waterhyacinth mats, where mats border open water, or in areas where extensive regrowth occurs (Center 1979, 1981a).

Description

47. Adult. The small and delicate adult (Figure 4a), is usually yellowish tan with brown markings. The wing span is 20 mm, with triangular fore wings varying in color from gold to brown. The broader hind wings are usually gold. Two wing spots are distinctive: a white spot centrally located on the fore wing and a dark spot centrally located on the hind wing. The body segments appear ringed because the posterior edges of segments are almost always white. Females are usually darker than males (Center 1979). Adults lack chewing mouth parts and do not feed on waterhyacinth.

48. Egg. The egg (Figure 4b) is small (0.3 mm), spherical, and creamy white. Eggs darken as they develop and appear black immediately prior to eclosion due to the dark head capsule of the developing larva.

49. Larva. The newly eclosed larva, which is approximately 1.5 mm in length, is brownish with darker spots. The head capsule is black or dark brown (Center 1981a). As the larva matures (Figure 4c), it is characterized

* Personal Communication, Donald Kenney, Abbott Laboratories, 1982.



a. Adult



b. Egg



c. Larva



d. Pupa

Figure 4. Life stages of *Sameodes*

by dark-brown to brownish-purple spots on the dorsal surface of a cream-colored body. When fully grown, the fifth instar larva is approximately 2 cm in length and has a dark orange head capsule (Center 1979). Only the larva feeds on waterhyacinth.

50. Pupa. *Sameodes* pupa (Figure 4d) are dark reddish brown or nearly black, with obtect morphology (appendages more or less glued to the body). The pupa is enclosed in a white silken cocoon in a waterhyacinth petiole.

Reproduction and life cycle

51. *Sameodes* is a multivoltine moth, producing as many as five generations in a year (DeLoach and Cordo 1978). Generation time is dependent on ambient temperatures, but may be as short as 21 days at 30°C. Reproduction is inhibited at 35°C. Average generation time is 27 to 30 days under greenhouse conditions (Center 1979).

52. *Sameodes* undergoes a complete metamorphosis with five larval molts. The following paragraphs briefly describe life cycle events. Center (1981a) provides a more detailed description.

53. Adult. Adults live a maximum of 7 days following emergence. Mating occurs soon after emergence, and the male usually dies immediately after mating. Females may oviposit for several days after mating, but most eggs are oviposited during the second night following emergence. Egg number varies greatly, but averages of 300 (DeLoach and Cordo 1978) to 450 (Center 1981a) are not uncommon. Eggs are often laid on portions of waterhyacinth leaf blades where the epidermis has been removed or damaged by other organisms, but oviposition may also occur on undamaged leaves.

54. Egg. Following oviposition, larval development in fertile eggs progresses rapidly. The egg darkens as the larval head capsule enlarges, and eclosion occurs in 4 to 7 days.

55. Larva. The newly emerged larva feeds on leaf blade tissues for a few hours following eclosion, after which it burrows into the petiole. The larva often moves to the plant crown and feeds on petiole epidermis prior to tunneling into the petiole. Once a larva enters the petiole, it feeds on internal tissues and grows rapidly. Intensity of feeding increases after each of the first four molts and major damage is inflicted by third through fifth instar larvae. Larval development is completed in 16 to 18 days.

56. Pupa. Pupation usually occurs in the mid-portion of a large, inflated petiole. The mature larva tunnels into the petiole and produces an

elliptical pocket or cavity. The larva also consumes tissues in an area adjacent to the petiole epidermis on the opposite side from its entrance tunnel. This serves as an exit point for the emerging adult, and appears as a round hyaline window (Figure 5). The larva lines the cavity and tunnel with silk and spins a white silken cocoon around itself. The last instar larval skin is molted and pupation begins, which requires 7 to 10 days. In dense populations, several pupae may occur in the same petiole (Figure 4). When the adult is fully developed, it emerges from the cocoon, crawls through the tunnel, and breaks through the hyaline window. It usually rests on the lower leaf surface for about an hour until its wings expand and dry.



Figure 5. Hyaline window (upper right) produced by *Sameodes* larva. Also note frass on centermost petiole

Characteristics of infestation

57. First instar larval feeding on leaf blades. Newly emerged larvae typically feed on the leaf blade in the portion where the eggs were laid. The feeding pattern is random and consists of removal of the epidermal tissues (Figure 6a). Careful examination of such areas may reveal tiny larvae, which are most easily recognized by the prominent dark head capsule. They may also occur at the petiole base, where they often feed on the epidermis of new leaves and associated leaf bract.

58. Small entry tunnels. Newly emerged larvae often tunnel into the petiole of the leaf on which they emerged. These entry tunnels (Figure 6b) are very small and often occur in the upper one third of the petiole. Tissues around the entry tunnels become necrotic or watery.

59. Large entry tunnels. Larger larvae move from one petiole to another. The entry tunnel made by these larvae (Figure 6c) will be much larger than those produced by the first instar larvae.

60. Wilting of leaf blades. As older larvae tunnel inside the petiole, they eventually consume sufficient vascular tissue that water movement to the leaf blade is obstructed. The leaf blade wilts rapidly when this occurs. Observation of a dried, green leaf blade is one of the most easily detected indicators of the presence of *Sameodes*. Since the larvae prefer newer leaves, wilted leaves are usually centrally occurring (Figure 6d).

61. Pupal windows. Hyaline windows (Figure 5), produced as an exit tunnel for emerging adults, are another indicator of the presence of *Sameodes*.

62. Frass. The excrement of *Sameodes* larvae may be found in petioles, on their surface, or in the crown of the rhizome. The frass (Figure 5) is reddish brown and usually occurs in masses. Although *Sameodes* frass has an odor, it may be distinguished from *Arzama* frass by the stronger odor and darker red color of *Arzama* frass. Because *Sameodes* prefers Stage I and Stage II plants, the above indicators will normally be found on smaller plants. However, they may occasionally be found on Stage III plants. Mature larvae are capable of tunneling into the thick petioles of Stage III plants, and these are sometimes used as pupation sites.

Host specificity

63. Extensive host specificity tests conducted on *Sameodes* in Argentina and in quarantine in the United States revealed that *Sameodes* feeding is limited to members of the Pontederiaceae. Although 12 of 46 potential host plant species were used as oviposition sites, larvae developed only on waterhyacinth, or infrequently on *Eichhornia azurea* (Swartz) Kunth and *Pontederia cordata* L. Population survival is dependent on the presence of waterhyacinth (DeLoach and Cordo 1978). Based on these studies, *Sameodes* was approved for field release in the United States in 1977.



a. First instar feeding



b. Small entry tunnels



c. Large entry tunnel



d. Wilted leaf

Figure 6. Characteristics of *Sameodes* infestation

Neochetina eichhorniae Warner

Taxonomy

64. *Neochetina eichhorniae* Warner (Order Coleoptera, Family Curculionidae), commonly known as the mottled waterhyacinth weevil, is one of six species of *Neochetina* that have been classified from the New World (DeLoach 1975). It has been collected from South America, Trinidad, Panama, and Mexico (O'Brien 1976).

Description

65. Adult. The adult weevil (Figure 7a) is 3 to 5 mm in length and is initially brownish gray, but becomes nearly black with age. The dorsal surface often has light-colored, nondistinct spots (mottles), which become obscure with age. Adults actively feed on both leaves and petioles, primarily at night.

66. Egg. The egg (Figure 7b) is whitish, slightly less than 1.0 mm in length, and slenderly ovoid. Although soft for 1 or 2 days, it soon becomes rigid.

67. Larva. The larva (Figure 7c) is uniformly white with a light-brown head capsule. The head capsule is smaller than that of *Neumodes*, and the body shape is scarabaeciform (grublike). Three stadia occur during larval development and mature larvae range in length from 7 to 10 mm with head capsules averaging 0.7 mm in width.

68. Pupa. Pupation occurs in the root system of waterhyacinth. The pupal case, which is light brown to black and probably chitinous, is covered by an interwoven mass of root hairs and is attached to the root system (Figure 7d).

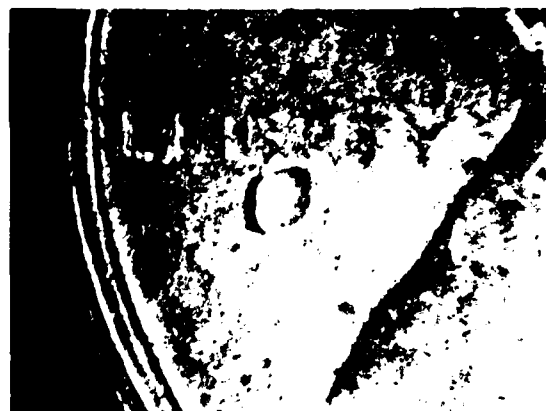
Reproduction and life cycle

69. *Neochetina eichhorniae* is multivoltine (two or three generations per year) and undergoes a complete metamorphosis (DeLoach and Cordo 1976a). The generation time ranges from 90 to 120 days. The following paragraphs describe the life cycle.

70. Adult. The newly emerged adult begins feeding on waterhyacinth leaf blades and petioles. Mating soon occurs and both sexes continue to actively feed for 3 to 4 months. The female oviposits individual eggs on the lamina of new leaves and ligules furled around the central bud. Oviposition is subepidermal and usually occurs in feeding spots.



a. Adult



b. Egg



c. Larva



d. Pupa

Figure 7. Life stages of *Neochetina eichhorniae*

71. Egg. The egg develops rapidly and eclosion occurs within 7 to 8 days. Egg development occurs within a temperature range of 20° to 35°C (DeLoach and Cordo 1976b).

72. Larva. The newly emerged larva penetrates the petiole in its upper one third and begins feeding on internal tissues. As the larva grows, feeding proceeds down the petiole, molting occurs, and development is nearly complete when the larva reaches the petiole base. A mature larva may tunnel into the plant crown and through the base of other petioles. When development is complete, the larva moves into the root system and penetrates a secondary root to its vascular tissue. Larval development requires 69 days.

73. Pupa. The developed larva produces a cocoon made from root hairs of waterhyacinth and secretes a pupal case around itself. The pupal stage requires about 30 days, after which the adult emerges. Successful completion of the pupal stage depends on the continued attachment of the pupal case to the waterhyacinth root. The pupa apparently receives oxygen from the plant through this attachment.

Characteristics of infestation

74. Feeding scars. The most obvious and easily detected indicator is the presence of feeding scars produced by adult weevils on the leaf blades (Figure 8a). Found primarily on the upper leaf surface, the feeding scars range from small nicks to lesions of 25 mm² (\bar{X} = 4.5 mm²). Characteristically, feeding scars penetrate the epidermis and several layers of mesophyll, but seldom extend through the lower leaf surface. In areas of dense weevil populations, individual leaves may have 500 or more feeding scars.

75. Girdled petioles. Adult weevils often girdle the petiole at its juncture with the leaf blade (Figure 8b). This may result in desiccation of the leaf blade, beginning at the apex.

76. Discolored petioles. Discolored areas produced by larval tunneling often occur in the petiole (Figure 8c). Usually elongate, these discolorations are especially evident in the lower one third of the petiole.

77. Rhizome damage. When the petiole is separated from the rhizome, evidence can often be found where large larvae have burrowed through the petiole into the rhizome (Figure 8d).

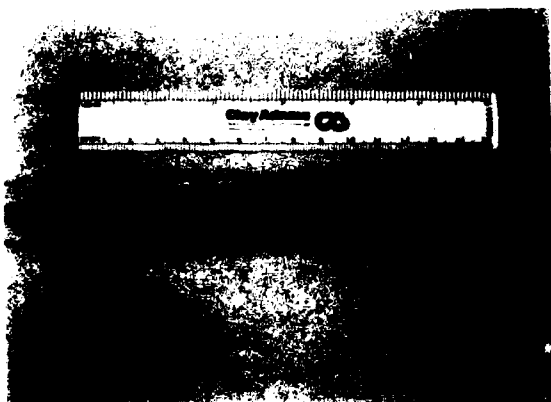
78. Pupal cases. Pupal cases usually occur immediately below the rhizome base and are difficult to locate because the pupal case is the same color as the surrounding roots (Figure 7d). Both adults and larvae actively



a. Feeding scars



b. Girdled petioles



c. Discolored petioles



d. Rhizome damage

Figure 8. Characteristics of *Neochetina eichhorniae* infestation

feed on waterhyacinth. Together, they are capable of significantly stressing the plant and may kill the plant when the weevil population density is of sufficient magnitude.

Host specificity

79. Although *N. eichhorniae* will feed and oviposit on *Zebrina*, *Brassica*, *Lactuca*, and a few other plants, waterhyacinth is by far the preferred species. The life cycle has been completed only on waterhyacinth. Based on studies by DeLoach and Cordo (1976a), it was concluded that *N. eichhorniae* was sufficiently host specific for introduction into the United States. Consequently, approval for its field release was obtained in 1972.

Neochetina bruchi Hustache

80. *Neochetina bruchi* (Figure 9), the chevroned waterhyacinth weevil, is a close relative of *N. eichhorniae*. The two species have similar native ranges and ecological niches, although *N. bruchi* can tolerate slightly colder temperatures than *N. eichhorniae* (DeLoach 1976).

81. The two species can be most easily distinguished by a broad, semi-circular white band (chevron) on the elytra of *N. bruchi*, which is absent on

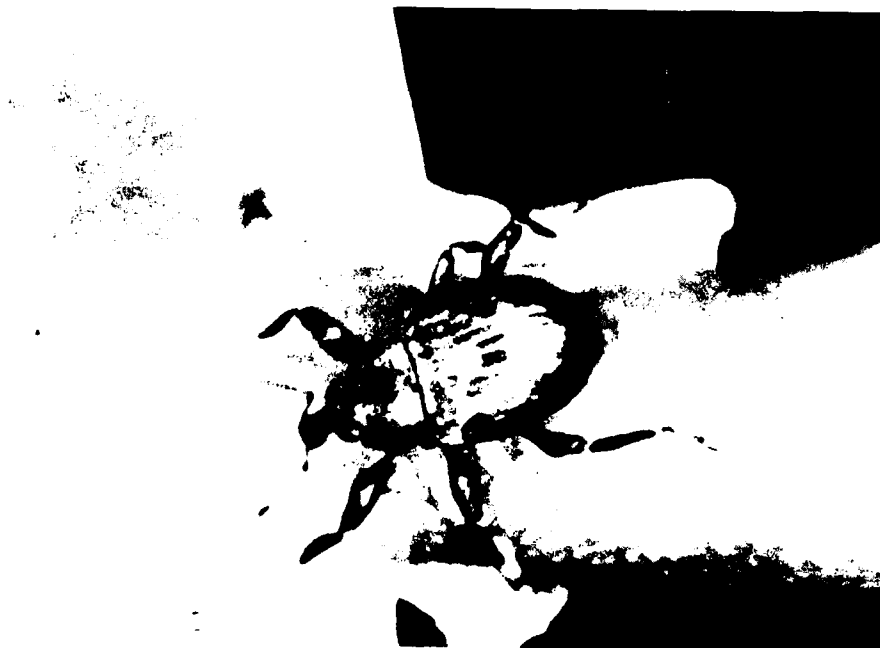


Figure 9. Adult *Neochetina bruchi*

N. eichhorniae (DeLoach and Cordo 1976a). Other stages in the life cycles of the two species are virtually indistinguishable, except to taxonomic experts.

82. Approval for field release of *N. bruchi* in the United States was obtained in 1974, and it was introduced into Louisiana by the LDWF in 1975 (Manning 1979). However, because *N. bruchi* was not encountered during the LSOMT studies, no further discussion of *N. bruchi* is warranted.

Arzama densa Walker

83. *Arzama densa* (Figure 10), a native noctuid moth that normally feeds on *Pontederia cordata*, has adapted to and is capable of completing its life

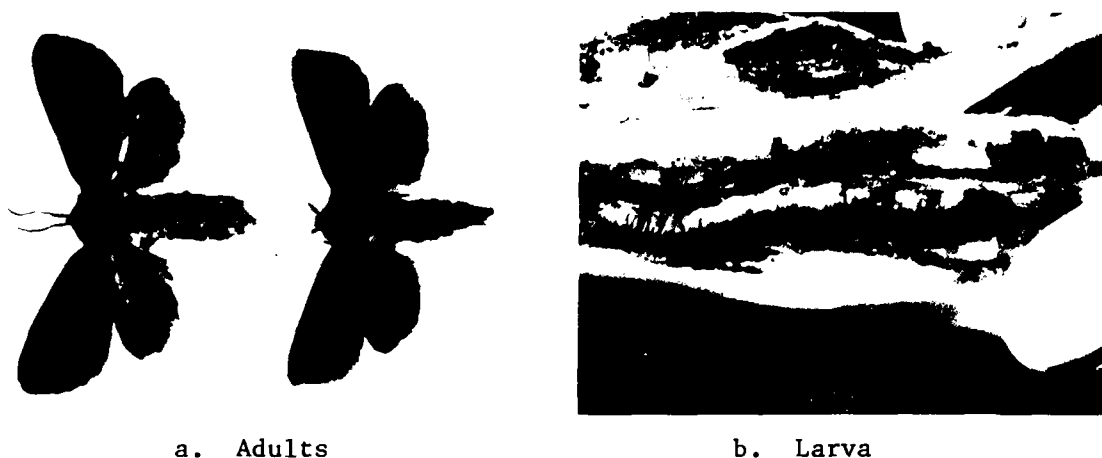


Figure 10. Adults and larva of *Arzama*

cycle on waterhyacinth. The life cycle and biology of *Arzama* have been documented by Center (1976). Although capable of locally damaging waterhyacinth, population development is sporadic and unpredictable. The highly mobile adults may fail to maintain a population at a given location through several generations. Larvae populations are severely impacted by several insect predators and are infected by viral diseases.

84. Recognizing the limitations of *Arzama* population development under field conditions, mass-rearing and release of artificially high numbers to augment naturally occurring populations was thought to be the only manner in which the moth could be effectively used. These efforts are summarized in Part IV.

85. Because *Arzama* occurs throughout Louisiana and is capable of producing significant local impacts on waterhyacinth, routine monitoring of *Arzama* populations at all study sites was deemed necessary.

Orthogalumna terebrantis Wallwork

86. *Orthogalumna*, the Argentine waterhyacinth mite, is a galumnid mite that occurs natively on waterhyacinth in South America. Since there is no documented evidence of intentional introduction of *Orthogalumna* into the United States, it was thought to have been introduced on waterhyacinths imported from South America.

87. The taxonomy, life cycle, and biology of *Orthogalumna* have been documented by Del Fosse (1975).

88. The presence of *Orthogalumna* is evidenced by intervascular tunnels in waterhyacinth leaves (Figure 11), resulting from feeding by the nymphs. These tunnels may often occur in most of the leaf blade and can most readily be observed when an infested leaf is held toward the sun. The major impact of *Orthogalumna* on waterhyacinth is the reduction of actively photosynthesizing leaf surface. However, the tunnels may also serve as points of entry for various weak pathogens such as *Acremonium zonatum* (Saw.) Gams.

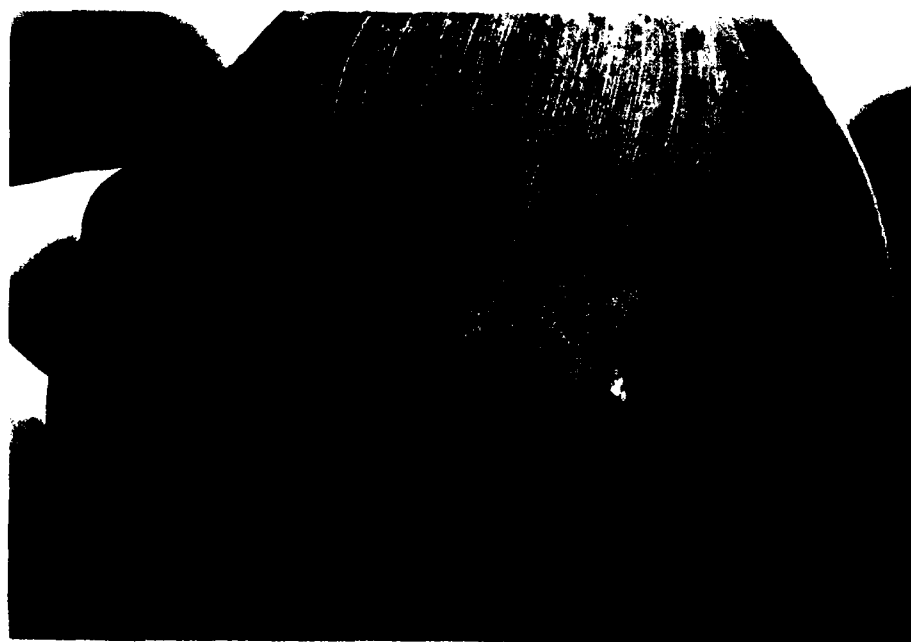


Figure 11. Tunnels produced by *Orthogalumna*

PART IV: PRELIMINARY STUDIES AND RELEASES

89. After the various species were discovered, evaluated for potential as biocontrol agents, and permission had been obtained for their release in the United States, a number of preliminary field studies were conducted to evaluate their effectiveness and/or determine methods for their use. Some studies were sponsored directly by the APCRP, but most were funded by LMN as part of the LSOMT. These studies are summarized in the following paragraphs.

Cercospora

90. The decision to include *Cercospora* in the LSOMT was based on the fact that Abbott Laboratories had developed a potentially commercial formulation of the fungus that could be mass applied. Together with promising results reported by the University of Florida (Conway, Cullen, and Freeman 1979), it appeared that *Cercospora* offered significant potential as a biocontrol agent. Preliminary studies conducted as part of the LSOMT prior to large-scale field releases are discussed below.

Application rate study

91. A replicated rate study was conducted in outdoor pools at WES to determine optimal *Cercospora* application rates (Theriot, Theriot, and Sanders 1981a). An application rate of 5×10^6 CFU (colony forming units) per square metre provided adequate infection of waterhyacinth plants.

Application equipment evaluation study

92. A test was conducted in a roadside canal near LaPlace, La., to evaluate two systems for application of the formulation (Theriot, Theriot, and Sanders 1981b). It was found that either application system could be used to effectively apply the formulation.

Sameodes

93. Subsequent to the 1977 approval for *Sameodes* field release, the APCRP funded the USDA to develop release methods and make field releases in Florida. The USDA monitored the dispersal and effectiveness of *Sameodes* on waterhyacinth.

Materials and methods

131. Establishment of study area. Since the northern end of the canal was open, a floating barrier consisting of 4-in. PVC pipe attached to 0.25-in.-diam steel cable was placed across the canal to prevent waterhyacinths from floating out of the study area. The resulting study area was 4.5 acres.

132. Sameodes releases. A site located approximately 100 m south of the berm was selected for a *Sameodes* release. This area was selected because the waterhyacinth population consisted of a fringe of small, bulbous-petioled (Stage I) plants that were better suited to *Sameodes* establishment than the larger Stage III plants found in the study area. Approximately 10,000 eggs and first instar larvae obtained from the USDA Aquatic Plant Management Laboratory (APML), Fort Lauderdale, Fla., were released in May 1979. Mr. Wiley Durden (APML) assisted in the release. The method for producing the *Sameodes* used in this release was described by Center (1981a). Leaves containing eggs and larvae were inserted into the center unfurled leaves of waterhyacinth plants (Figure 16). This procedure both supported and protected the eggs until the larvae emerged. A second release made in June 1980 consisted of approximately 1000 eggs and first instar larvae released approximately 50 m north of the berm by the same method used for the first release.

133. Application of *Cercospora*. The same *Cercospora* formulation used in the field application rate study (paragraph 110) was used in this study. The formulation contained approximately 5.0×10^5 CFU/g. A fixed-wing aircraft (Figure 17) with a conventional microfoil boom system was used for the application. The application boom was equipped with 0.012 nozzles with No. 46 orifice disc inserts. Screens on the pump and nozzles were removed to prevent the formulation from clogging the system. A total of 160 lb of formulation was applied on 8 May 1980 at a rate of 35.7 lb/acre (1×10^6 CFU/g). Due to the relatively large volume of water required for formulation suspension (260 gal of water/80 lb of formulation), it was necessary to divide the formulation into two portions and apply each portion separately. A surfactant, Ortho X-77, was added to each batch of formulation at a rate of 1.9 ml/gal. The pilot maintained an average altitude of 10 ft over the study site during the application (Figure 17). The period between applications was

Site selection and description

129. Several potential study sites were evaluated using the following criteria:

- a. Minimum of 4 acres of uniform waterhyacinth population.
- b. Minimal water flow through the area.
- c. Relatively isolated, low-use area.
- d. Minimum likelihood of herbicide applications.
- e. Sufficient water depth to preclude dewatering.

130. The site (Figure 15) selected for the study was a canal (T18S, R16E) extending northward from Lake Theriot in Terrebonne Parish, Louisiana, to the Intracoastal Waterway. The canal was blocked on the southern edge of the study area by a berm, and water flow through the canal was minimal. During infrequent periods of high flows, water flowed from north to south through the adjacent marsh, but emergent marsh vegetation prevented movement of waterhyacinth out of the study area. The berm effectively prevented boat traffic through the area. The study site contained a uniform-sized population of waterhyacinth that covered the entire water surface. Due to its remoteness and low use, the site had not been sprayed with herbicides in recent years.



Figure 15. Lake Theriot study site immediately prior to *Ceratospora* application

junction of the leaf blade and petiole. The principal effect of adult weevils on individual plants appeared to be a major reduction in leaf surface area available for photosynthesis. Removal of the upper epidermis also disrupted the water balance in the leaves, causing internal tissues to become desiccated. Since most stomata are located on the upper leaf surfaces, feeding by the adult weevils probably also disrupted the normal gas exchange process. Effects induced by larval feeding were even more pronounced. Larval damage in the lower portion of petioles and the rhizome was so severe by July that collection of plant samples became very difficult. The petioles often separated from the rhizome as plants were removed from the water. These plants often had as many as four late instar larvae at the base of petioles and in the rhizome. Larval feeding and the resulting tissue necrosis combined to effectively disrupt translocation of water and nutrients from leaves to the rhizome and roots. In addition, larval feeding also damaged or destroyed lateral meristems in the rhizome from which stolons are normally produced. This probably contributed to the reduced daughter plant production.

Conclusions

127. Conclusions of this study were:

- a. A significant decline of waterhyacinth in all test plots was due primarily to feeding activity by a dense *Neochetina* population.
- b. Although *Cercospora* became established in the test plots, it did not contribute significantly to the observed decline in the waterhyacinth population.
- c. The effects of *Neochetina* on the plant population precluded establishment of the optimum treatment rate for field applications of *Cercospora*.
- d. *Neochetina* is an effective biological agent for the control of waterhyacinth, and is capable of not only stressing waterhyacinth, but also of effecting a significant reduction in the plant population.

Neochetina, *Sareodes*, and Spring Application of the Original *Cercospora* Formulation

Purpose

128. The purpose of this study was to demonstrate the effects of a combination of *Neochetina*, *Sareodes*, and a spring application of the original *Cercospora* formulation on waterhyacinth in southern Louisiana.

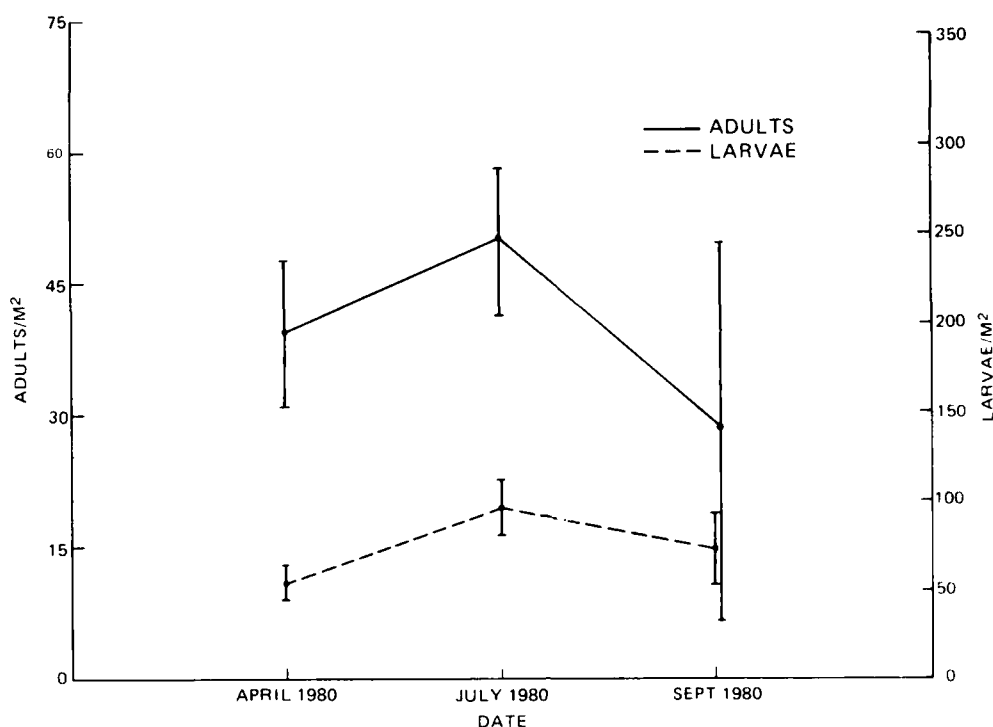


Figure 14. Mean numbers of *Neochetina* adults and larvae at Amelia study site. Vertical bars represent two standard errors of means

was isolated from plant tissues in both July and September, few characteristic symptoms of *Cercospora* pathogenicity were ever observed. The marked increase in pathogen damage in September was attributed to weak facultative pathogens and saprophytes that were adventive on the severely stressed plants. This tenet is supported by the decline of waterhyacinth in all test plots (including untreated controls), and the increase in pathogen damage ratings in control plots as well as those receiving applications of *Cercospora*. Thus, pathogen damage probably contributed to the decline in the plant population, but was not the primary factor effecting the observed decline.

126. *Neochetina*. This study strongly implicated *Neochetina* as the primary factor responsible for the rapid decline of waterhyacinth. A well-established *Neochetina* population was already present prior to the study. As the season progressed, feeding activity by high numbers of adults and significant increases in larval numbers severely stressed the plant population. Intense feeding by adult weevils had destroyed most of the upper epidermis of nearly all leaf blades by July. They also girdled most petioles at the

in April to 22.2 cm in July. Daughter plant production declined significantly from a mean of $31.3/\text{m}^2$ in April to $7.2/\text{m}^2$ in July.

121. Pathogen damage. Mean pathogen damage per leaf for all treatments, including untreated controls, is presented in Table 2. Mean values for all plots treated with *Cercospora* declined in July as compared to pretreatment values in April, while the mean value for untreated controls increased slightly. Pathogen damage increased on all treated plots in September, but too few plants remained in the untreated control plots to allow sampling. Increased pathogen damage in September was not attributable to *Cercospora* because the fungus could only rarely be isolated from samples.

122. Neochetina. The mean number of *Neochetina* adults increased from $39.4/\text{m}^2$ in April 1980 to $50.0/\text{m}^2$ in July 1980 and declined to $28.3/\text{m}^2$ in September 1980 (Figure 14). The mean number of *Neochetina* larvae increased from $54.5/\text{m}^2$ in April 1980 to $97.6/\text{m}^2$ in July, and then declined to $73.2/\text{m}^2$ in September 1980 (Figure 14); however, the differences were not significant. The mean numbers of both adults and larvae per plant were higher in September than in April due to the presence of fewer waterhyacinth plants in September.

123. Other organisms. *Arzama* occurred in the test plots, but at very low population levels. Only five plants examined in July showed evidence of *Arzama* feeding damage, and only one larva was found in September.

Discussion

124. The waterhyacinth population declined rapidly. Percent cover and plant biomass (weighted by percent cover) were greatly reduced in September in all test plots (including controls), and the remaining plants were much shorter than normally encountered in Louisiana in September. Plant density was much lower in September than in April, but daughter plant production was very low in September. This is reverse of the normal pattern observed for waterhyacinth growth in Louisiana. Percent cover, biomass, and plant height normally peak in September, with an associated decrease in plant density. Daughter plant production normally increases sharply as plant density and percent cover decrease, but this pattern did not occur. The atypical growth pattern clearly indicated that one or more extrinsic factors were causing a significant decline in the waterhyacinth population.

125. Pathogen damage. Pathogen damage increased significantly on waterhyacinth leaves and petioles in all test plots (Table 2). Although *Cercospora*

117. The mean percentage of surface area coverage of waterhyacinth in each test plot was determined by averaging estimates of three observers. The mean number of waterhyacinth plants per quadrat was calculated for each plot on each sampling trip by averaging the number of plants in the four sampled quadrats. Weighted means were calculated by multiplying the plot mean by the decimal fraction of surface area coverage. Biomass data were analyzed in a similar manner. Plot means for the number of daughter plants and plant height were calculated, but weighted means were not determined. Analysis of variance (ANOVA) was used to determine whether means for each parameter varied significantly among treatments and sampling periods.

118. Average pathogen damage per leaf in each quadrat was calculated by summing disease index values for all leaves on each plant and dividing by the total number of leaves. Plot means were determined by summing quadrat means and dividing by the number (four) of quadrats. Mean pathogen damage per test plot was averaged across treatment plots for each sampling date. ANOVA was used to determine whether pathogen damage differed significantly among treatments and sampling trips.

119. Mean numbers of *Neochetina* adults and larvae per square metre were calculated for each quadrat. Resulting means were averaged for each plot and among plots for each sampling trip. ANOVA was used to determine whether mean numbers of *Neochetina* adults and larvae per plant and quadrat varied significantly among sampling trips.

Results

120. Waterhyacinth. The waterhyacinth population in all test plots (including controls) declined rapidly following treatment (Table 1). Percentage of surface area covered by waterhyacinth decreased from an average of 89.9 percent in April to 33.6 percent in July and 10.2 percent in September. Plant density decreased significantly* from a mean of $116.7/m^2$ in April to $40.5/m^2$ in September. When weighted by percent cover, mean plant density declined from $104.8/m^2$ in April to $4.1/m^2$ in September. Although plant biomass declined in a similar manner, the differences were not significant. When weighted by percent cover, mean biomass declined from $10.6\text{ kg}/m^2$ in April to $0.6\text{ kg}/m^2$ in September. Mean plant height increased significantly from 8.0 cm

* All references to significant or significance represent statistical significance at the $p < 0.05$ level.

NUMERICAL RATINGS AND SYMPTOMS

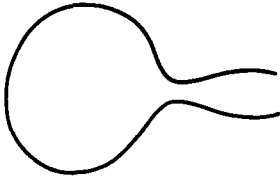
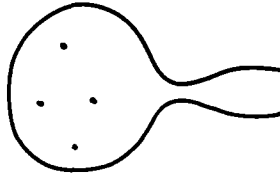
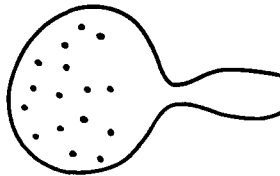
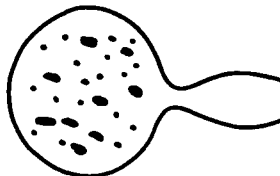
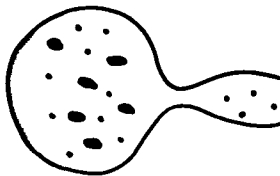
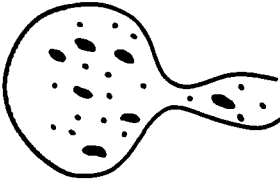
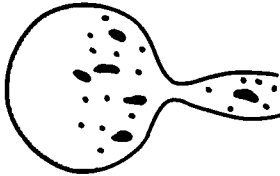
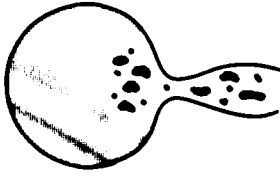
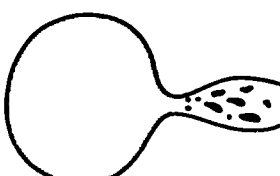
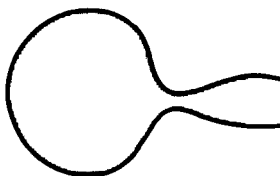
0	1	2	3	4
NO SPOTS ON LEAF OR PETIOLE.	1 TO 4 SPOTS ON LEAF, NO PETIOLAR SPOTTING.	LESS THAN 25 PERCENT OF LEAF SURFACE WITH SPOTS, NO COALESCENCE OR PETIOLAR SPOTTING.	LESS THAN 50 PERCENT OF LEAF SURFACE WITH SPOTS, SOME COALESCENCE, NO PETIOLAR SPOTTING.	LESS THAN 25 PERCENT OF LEAF SURFACE WITH SPOTS, COALESCENCE, SOME TIP DIEBACK AND PETIOLAR SPOTS.
				
5	6	7	8	9
LESS THAN 50 PERCENT OF LEAF SURFACE WITH SPOTS, COALESCENCE, 10 PERCENT TIP DIEBACK, PETIOLE SPOTTING.	LESS THAN 75 PERCENT SPOTS, COALESCENCE, 30 PERCENT TIP DIEBACK, INCREASING PETIOLE SPOTTING.	GREATER THAN 75 PERCENT SPOTS, COALESCENCE, 60 PERCENT TIP DIEBACK, COALESCING SPOTS ON PETIOLE.	DEAD LEAF BLADE, PETIOLE GREEN, BUT HEAVILY SPOTTED.	DEAD LEAF BLADE AND PETIOLE (SUBMERGED).
				

Figure 13. Pathogen damage index rating system

of the spray mixture, one half of the total volume was applied across the plot in one direction, and the other half was applied at right angles to the first application. The sky was overcast during application and remained overcast until nightfall. Ambient temperatures during application were 24° to 27°C. Wind velocity was less than 10 mph from a southwesterly direction.

111. Sampling procedure. The procedures discussed below were used for sampling waterhyacinth, pathogen damage, and arthropod species.

112. The percentage of the test plot surface covered by waterhyacinth was visually estimated by three observers prior to sampling. All waterhyacinth plants were collected from four randomly located 0.25-m² (0.5 m × 0.5 m) quadrats in each test plot, and samples were placed in plastic bags for analysis. Height of the centermost plant in each quadrat was recorded prior to removal of the plants from the quadrat. Plants from each quadrat were placed in a wire basket, allowed to drain for 1 min, and weighed to the nearest gram. The number of mature plants and daughter plants was recorded separately for each quadrat. Daughter plants consisted of individuals with one or more unfurled leaves, no functional roots, and with the plant still attached to the parent plant by a stolon.

113. Five waterhyacinth plants from each quadrat were randomly selected for assessing pathogen damage. Each leaf of these plants was examined and a disease rating index value was assigned, using categories shown in Figure 13. Samples were collected from selected leaves for laboratory reisolation of *Cercospora*.

114. Each of the five plants used for assessing pathogen damage were examined for *Neochetina* adults and larvae, *Arzama* larvae, and other arthropod species (e.g. *Orthogalumna*). For each species, the number of individuals of each life stage was recorded for each plant.

115. Sampling schedule. All test plots were sampled on 17-18 April 1980 prior to application of the formulation. Posttreatment sampling was conducted on 12 July and 30 September 1980. Sampling was discontinued after September due to insufficient numbers of waterhyacinth plants in test plots to obtain valid samples.

116. Data analysis. Resulting data were analyzed as discussed in the following paragraphs.

approximately 2 miles north of the intersection of Louisiana Highway 398 with Louisiana Highway 622. The canals were bordered by deep cypress-tupelo swamps on one side and the highway embankment on the other. Dense fringes of willows occurred along the highway embankment. The canals were uniformly covered by mats of waterhyacinth along their entire length, with other plant species (e.g. bidens, pennywort, and *Habenaria repens*) occasionally interspersed in the mats.

Materials and methods

109. Establishment of test plots. Twelve 336-m² test plots were established. The test plots were alternated on either side of the highway and separated by a distance of 0.4 mile. Each plot was delimited by barriers constructed of 4-in.-diam polyvinyl chloride (PVC) pipe bound to 0.25-in. steel cable, and positioned across the canal on both ends of the plot. The cable length was sufficient to maintain the barrier at the water surface as the water level fluctuated. Plot dimensions varied according to canal width, but the plot size was uniform.

110. Application of *Cercospora*. The *Cercospora* formulation developed by Abbott Laboratories consisted of thick-walled vegetative cells dispersed in a wettable powder medium that had been sufficiently milled to pass through a 50-mesh screen. Previous studies (Theriot, Theriot, and Sanders 1981a) indicated that an application rate of 5.0×10^6 CFU/m² provided an acceptable level of infectivity. To determine the optimum inoculum rate for field use, application rates of 4×10^4 , 4×10^5 , and 4×10^6 CFU/m² were tested. Treatments, including a control consisting of spray mix without the *Cercospora* formulation, were randomly apportioned to the test plots, and each treatment was replicated three times. All plots were treated on 19 April 1980, beginning with control plots and proceeding with 4×10^4 , 4×10^5 , and 4×10^6 CFU/m² applications. The application equipment consisted of a John Beam Roadside R20 Pump, a high-pressure piston pump (150 psi), with a 100-ft hose attached to a John Beam Deluxe Spray Master adjustable spray gun. A total of 45 gal of spray mixture was applied to each test plot. A surfactant, Ortho X-77, was used in all treatments at a rate of 0.15 ml/m² (50 ml per plot). Water used for all applications was obtained from a nearby bayou. The spray gun was adjusted to deliver droplet-sized particles. To ensure uniform application

Cercospora Field Application Rate Study

Purpose

106. The purpose of this test was to determine the range of application rates that provides optimum infectivity of *Cercospora* on waterhyacinth under field conditions.

Site selection and description

107. Site criteria. Potential study sites in southern Louisiana were evaluated by applying the following criteria:

- a. Uniform waterhyacinth population.
- b. Site configuration conducive to establishment of 12 test plots separated by a sufficient distance to preclude cross-contamination.
- c. Sufficient water depth to preclude dewatering of test plots.
- d. Unlikelihood of herbicide applications during the study.

108. Study site. A study site (Figure 12) conforming to the above criteria was selected near Amelia in Assumption Parish, Louisiana. The site (T155, R14E and 15E) consisted of deep roadside canals extending 5 miles on both sides of Louisiana Highway 398. The southern end of the site was located



Figure 12. Test site for *Cercospora* field application rate study prior to treatment

PART V: LARGE-SCALE FIELD TESTS

Test Design

104. The original test design for the LSOMT included both replicated and unreplicated tests (Sanders et al. 1979). These tests were to demonstrate the effectiveness of different combinations of biological agents in controlling waterhyacinth when applied at a scale comparable to operational situations. Due to management considerations, only the following unreplicated tests were initially to be conducted:

- a. *Cercospora* applied in spring.
- b. *Cercospora* applied in fall.
- c. *Cercospora* and *Sameodes*.
- d. Multiple applications of *Cercospora*.
- e. *Sameodes*.
- f. Combination of all biocontrol agents.

Because *Neochetina* was so widely distributed on waterhyacinth in Louisiana when the tests were initiated, it was included as a test organism in all tests.

105. Various factors resulted in further modification of the series of tests to be conducted. Due to changes in the *Cercospora* formulation, it was necessary to conduct a field application rate study. The limited availability of the *Cercospora* formulation resulted in deletion of the fall application test, and subsequent changes in the formulation made an additional spring application of *Cercospora* imperative. The following large-scale demonstration tests were actually conducted:

- a. *Cercospora* field application rate study.
- b. *Neochetina*, *Sameodes*, and spring application of the original *Cercospora* formulation.
- c. *Neochetina* and spring application of a modified *Cercospora* formulation.
- d. *Neochetina* and *Sameodes*.
- e. Establishment, dispersal, and distribution of *Sameodes*.

Each of these tests will be discussed in the following sections.

Summary

103. When the large-scale demonstration tests were initiated, *Cercospora* application rates and systems had been determined, *Sameodes* had been successfully established on waterhyacinth in Florida and methods for its release had been developed, and *Neochetina* was well established on waterhyacinth throughout Louisiana. Both *Arzama* and *Orthogalumna* also occurred on waterhyacinth throughout Louisiana. Thus, a decision was made to proceed with the large-scale evaluation of these species, used alone and in various combinations, for control of waterhyacinth in Louisiana.

98. *Neochetina* was well established on waterhyacinth throughout the state by the inception of the LSOMT, and it was difficult to find waterhyacinth populations that were not infested. However, the site at Sorrento was the only instance in which the weevils had been observed to significantly impact waterhyacinth populations.

Arzama

99. Based on studies by Center (1976), it was concluded that *Arzama* could only be effective as a biological agent if the moth could be mass reared and released during early spring to augment field populations. Its potential effectiveness was based on the fact that *Arzama* severely damages waterhyacinth plants; a single larva is capable of destroying the crown of several plants.

100. A study conducted by the USDA Southern Weed Science Laboratory, Stoneville, Miss., resulted in the development of a method for producing large numbers of *Arzama* larvae (Baer and Quimby 1980).

101. Using larvae produced at the USDA-Stoneville laboratory, a small-scale field test was conducted in a roadside canal at Norco, La., to test the concept of augmenting field populations of *Arzama*. Details of this study are presented by Cofrancesco (1982). Although the mass release of *Arzama* was found to be possible, its impacts on waterhyacinth were insufficient to reduce the plant population. The high mobility of adults precluded development of increased populations of *Arzama* on the site during subsequent generations.

102. A significant problem in developing a mass-rearing capability of *Arzama* was the period required for rearing newly emerged larvae to the third instar stage. This approach required large quantities of food material and occupied considerable laboratory space for long periods. Subsequently, a method was developed for producing large quantities of *Arzama* eggs, thereby alleviating problems associated with larval rearing (Baer and Quimby 1980). A small-scale field test was conducted in 1981 at Lake Salvador, Louisiana, to determine if significant field populations of *Arzama* could be established by releasing eggs. However, the release of eggs did not result in sufficient populations of *Arzama* to significantly impact waterhyacinth. Consequently, *Arzama* was excluded from further consideration in the LSOMT, except for monitoring its naturally occurring population levels at test sites.

94. Center (1981a) described several useful methods for conducting *Sameodes* field releases. All methods were found to be successful, but a method for releasing large numbers of larvae was to be most effective. Pupae were collected and the resulting adults were mated in Petri dishes containing a portion of a waterhyacinth leaf blade in which the upper epidermis was partially removed. The gravid female oviposited on the leaf and the eggs eclosed in 5 to 7 days, resulting in large numbers of first instar larvae. By synchronizing the population and carefully timing releases, field releases could be planned to coincide with egg eclosion.

95. Center (1981a) released *Sameodes* at 21 locations in Florida, and the moth became established in 17 sites and rapidly dispersed to surrounding areas. He found that *Sameodes* may disperse at a rate of 30 miles per month. The moth had spread to waterhyacinth in most areas in the lower two thirds of the state, and was well established as far north as the Florida-Georgia border by 1982. Because *Sameodes* was in a dispersal phase during most of the study, few instances of significant reductions in the waterhyacinth population were noted (Center 1981a).

Neochetina

96. Following approval in 1973 to release *Neochetina*, it was introduced on waterhyacinth populations throughout the southeastern states. The initial *Neochetina* releases in Louisiana were made in 1974 when the LDWF released approximately 200 adult weevils in each of five locations. Populations of weevils in these nursery areas were sufficient by 1976 to allow initiation of a state-wide release program (Manning 1979). During 1976 and 1977, the LDWF and LMN released a total of 158,026 weevils on waterhyacinth throughout the state. Most of the released insects were *N. eichhorniae*, but *N. bruchi* was released at some locations.

97. There was evidence as early as 1977 that *Neochetina* was significantly impacting waterhyacinth in Louisiana. The waterhyacinth population at Sorrento (Ascension Parish), one of the original nursery areas, was eliminated. Manning (1979) ascribed this effect to large weevil populations combined with especially severe winters during 1976 and 1977.



Figure 16. Release of *Sameodes* (eggs and first instar larvae) at the Lake Theriot study site in May 1979



Figure 17. Application of *Cercospora* at the Lake Theriot study site in May 1980

approximately 1 hr. All plants in the study area were wetted during each application, and the period between applications allowed the formulation applied on the first trip to dry. Individual waterhyacinth plants examined from several locations in the study area immediately following application contained numerous formulation particles on the leaves. Wind velocity during application was less than 10 mph, and overcast conditions prevailed immediately following the application. Ambient temperatures during the application ranged from 26° to 28°C.

134. Sampling procedure. Sampling was conducted in May, July, and October of 1980, and in April, July, and September of 1981. The following paragraphs discuss procedures used for sampling the waterhyacinth population, degree of pathogen damage, and arthropod species.

135. For waterhyacinth population, six randomly selected sampling points were chosen in the site. Each point served as the center of a circular (25-ft radius) sampling area. Locations for five 0.25-m² (0.5 m by 0.5 m) quadrats were identified in each sampling area by randomly selecting compass headings and distances (1-ft intervals) along the selected compass headings. All waterhyacinth plants in each quadrat were removed, placed in a plastic bag, and transported to shore. Watershoes were used for sampling to prevent compaction of plants by the airboat. Thirty quadrats were sampled on each sampling trip. The first sampling trip was conducted immediately prior to application of the formulation. Data recorded for waterhyacinth included percent cover (total area), biomass, density, height, number of leaves, and number of daughter plants. Biomass was determined by placing all plants from each quadrat into a wire basket, allowing 1 min for surface water to drain, and recording weight to the nearest gram. Plant density was determined by removing daughter plants (paragraph 85) and separately counting the mature plants and daughter plants in each sample. The heights (centimetres) and number of leaves on the centermost plant in each quadrat were recorded.

136. The degree of pathogen damage on each leaf of five plants per quadrat was assessed using the disease rating index (Figure 13). Plant tissues were randomly selected for laboratory reisolation of *Cercospora*.

137. For arthropod species, the numbers of the various life stages and damage produced by *Neochetina*, *Sameodes*, and *Arzama* on the sampled plants were recorded as follows:

- a. Neochetina. All plants in each quadrat were examined for *Neochetina* adults and larvae. The number of feeding scars produced by adult *Neochetina* was assessed for each leaf of two plants from each quadrat by using the following feeding index:

<u>Feeding Class</u>	<u>Number of Feeding Scars</u>
0	0
1	1-50
2	51-100
3	101-200
4	>200

Representative samples of adult *Neochetina* were collected for identification.

- b. Sameodes. All plants in each quadrat were examined for *Sameodes* larvae and pupae and damage produced by larvae.
- c. Arzama. All plants in each quadrat were examined for *Arzama* larvae, pupae, and/or damage produced by larvae.
- d. Orthogalumna. Each leaf of two plants from each quadrat was examined for *Orthogalumna* tunnels. A rating scale of 0 to 2 was used to characterize the degree of infestation, in which 0 = absent, 1 = ≤ 50 percent of the leaf blade with tunnels, and 2 = > 50 percent of the leaf blade with tunnels.

138. Data analysis. Percent cover of waterhyacinth in the study area was estimated by three observers and averaged. Mean values for all other parameters in each sampling area were determined. Sampling area means were averaged to produce overall means for each parameter for each sampling date. ANOVA was used to determine whether overall means varied significantly among sampling dates.

139. Quadrat means for pathogen damage per leaf were calculated by summing pathogen ratings for all leaves on five waterhyacinth plants in each quadrat and dividing by the total number of leaves sampled. Quadrat means were averaged to determine mean pathogen damage per leaf for each sampling date. ANOVA was used to determine whether or not the degree of pathogen damage varied significantly among sampling dates.

140. Means for *Neochetina* adults and larvae per square metre and *Sameodes* and *Arzama* larvae and pupae per square metre were calculated for each sampling date. Mean numbers of *Neochetina* adults and larvae per square metre were weighted by plant density. A mean index value for *Neochetina* feeding scars per leaf was calculated for each sampling date. ANOVA was used to determine whether calculated means varied significantly among sampling dates.

Mean index values of *Orthogalumna* tunneling per leaf were calculated for each sampling date.

Results

141. Waterhyacinth population. Percent cover of waterhyacinth in the study area remained at 100 percent throughout the study (Table 3). Mean biomass increased significantly during midsummer of 1980, decreased to its lowest level in April 1981, and increased significantly during the summer of 1981 (Table 3). However, biomass values for July and September of 1981 were significantly lower than for the same periods during 1980. Mean plant density (Table 3) decreased significantly during the 1980 growing season, increased early in the 1981 growing season, and then declined significantly during late summer of 1981. Plant densities during the spring of 1981 were significantly lower than for the same period of 1980. Mean plant height (Table 3) increased in July 1980 but did not increase significantly during the rest of the growing season. Plant height increased throughout the 1981 growing season, but the plants were significantly smaller in September 1981 than in October 1980. Daughter plant production (Table 3) declined significantly during the summer of 1980, increased significantly during the spring of 1981, and then decreased significantly during the summer of 1981. Daughter plant production was significantly greater in July of 1981 than during the same period of 1980.

142. Pathogen damage. Mean pathogen damage (Figure 18) increased significantly during late summer of 1980, decreased in the spring of 1981, then increased significantly during the 1981 growing season. Mean pathogen damage was significantly greater in July and September of 1981 than for the same periods during 1980. *Cercospora* was reisolated from waterhyacinth tissues on all posttreatment sampling dates, and symptoms of *Cercospora* damage were especially abundant in October 1980, and July and September of 1981.

143. Arthropod species. Only *Neochetina* occurred at sufficient population levels to affect the waterhyacinth population. The mean number of *Neochetina* adults/m² (Figure 19) was 0.4/m² in May 1980, increased significantly to 6.7/m² in October 1980, and reached a peak of 61.9/m² in July 1981. Means for adults were significantly higher in 1981 than for 1980 on all sampling dates. The mean number of *Neochetina* larvae (Figure 19) increased significantly from May 1980 (5.4/m²) to July 1981 (312.8/m²), but declined significantly during late summer of 1981 to 83.2/m². Means for all 1981

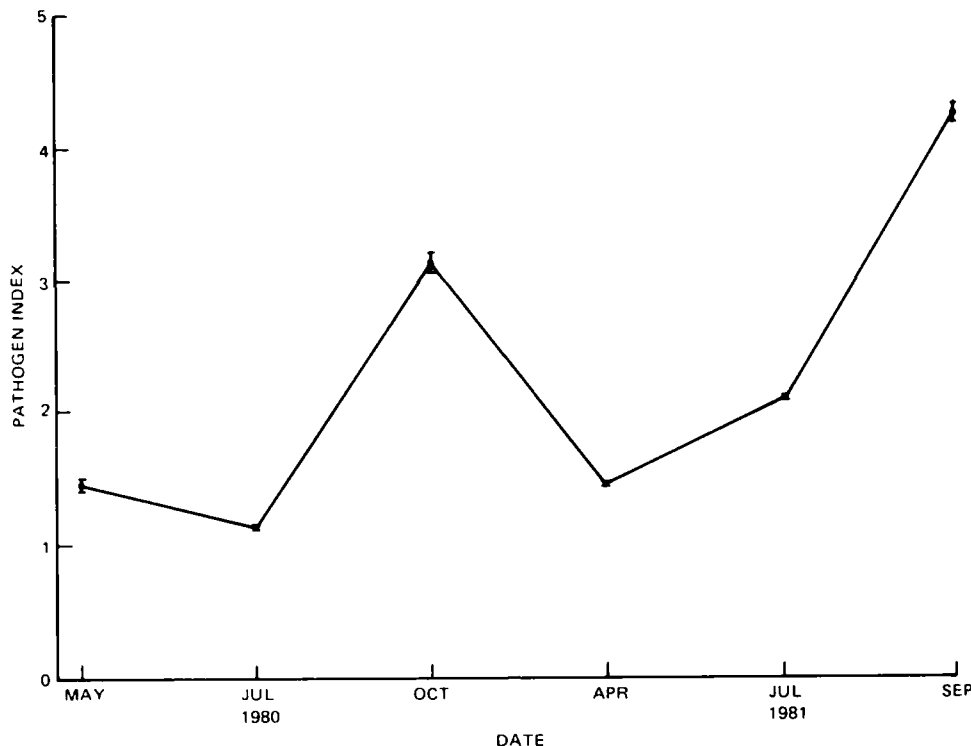


Figure 18. Mean pathogen damage index values per leaf for Lake Theriot study site. Vertical bars represent two standard errors of means

sampling dates were significantly higher than for the corresponding dates in 1980. Mean values for *Neochetina* feeding scars per leaf (Figure 20) increased significantly during 1980, decreased in April 1981, and significantly increased to a peak of 3.31 in September 1981. An index value of 3.31 is approximately equivalent to 133 feeding scars per leaf. Mean values for all 1981 sampling dates were significantly higher than for the corresponding periods during 1980. No *Sameodes* larvae or pupae were found during any sampling period, and no evidence of its presence was found anywhere in the study area. Means for *Arzama* larvae/m² and index values for *Orthogalumna* tunnels/leaf were low for all sampling dates (Table 4).

Discussion

144. Waterhyacinth population. The pattern of biomass production and plant density at Lake Theriot was generally characteristic of waterhyacinth population development in southern Louisiana. Biomass production normally increases until late summer, remains at a high level until frost, and declines

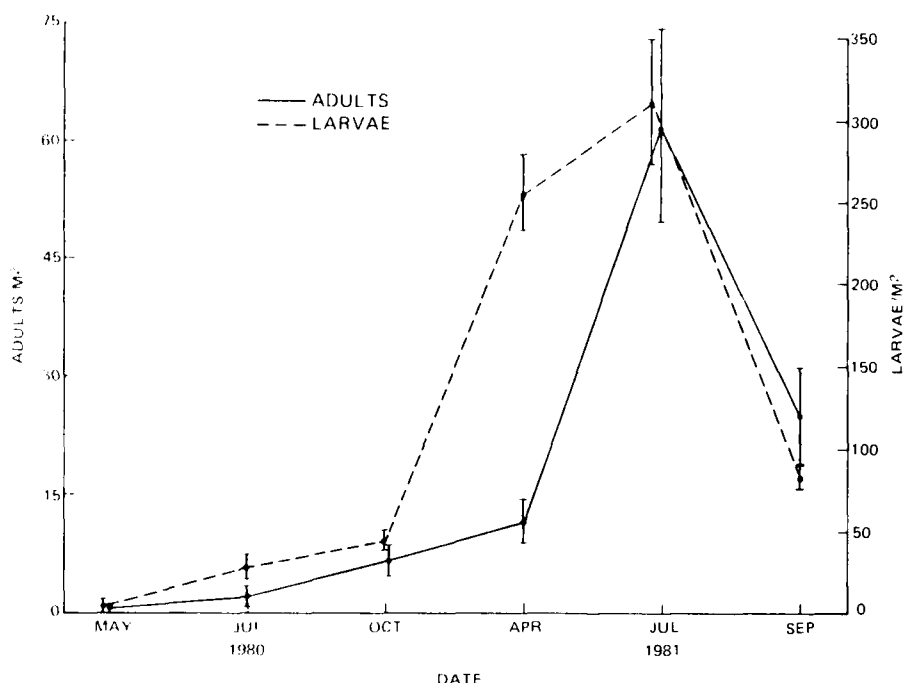


Figure 19. Mean numbers of *Neochetina* adults and larvae/m² at the Lake Theriot study site. Vertical bars represent two standard errors of means

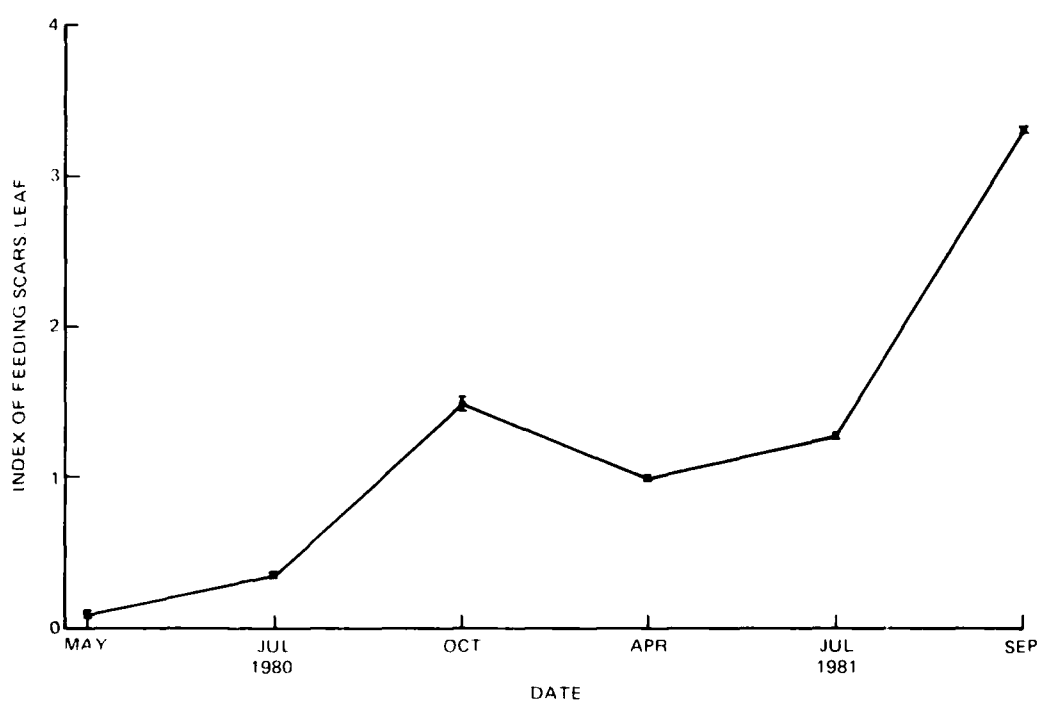


Figure 20. Mean values of feeding scars/leaf by adult *Neochetina* at the Lake Theriot study site. Vertical bars represent two standard errors of means

during the winter months to its lowest point at the onset of the next growing season. Plant density normally peaks in May, as a result of maximal daughter plant production during March and April. However, two significant variations in biomass production occurred during the study. Biomass decreased significantly from July to October 1980, which coincided with a significant decline in plant density during this period. Also, biomass values during all 1981 sampling dates were significantly lower than the means for the corresponding 1980 sampling dates. For example, mean biomass was 58 percent lower in July 1981 than in July 1980. Although plant densities exhibited the normal pattern for waterhyacinth in Louisiana, plant densities in the spring of 1981 were approximately 50 percent lower than in 1980. However, plant densities for July 1981 were not significantly different than values for July 1980. Although percent cover remained at 100 percent during the study, changes in plant biomass and density suggested that one or more factors were significantly impacting the waterhyacinth population. Herbicide applications and dewatering were ruled out as potential factors influencing the observed changes because neither occurred during the study.

145. Pathogen damage. The degree of pathogen damage increased significantly during late summer of 1980, and much of the damage was attributed to *Cercospora*. Symptoms of *Cercospora* were observed on waterhyacinth plants by July 1980 in the most sheltered portions of the area, particularly in portions protected by overhanging vegetation. However, few typical *Cercospora* symptoms were observed in the center of the study area. This was probably due to high ambient temperatures that inhibited the growth of *Cercospora*. Pathogen damage had increased significantly by October 1980, and much of the damage was typical of that produced by *Cercospora*. Symptoms were especially abundant on older, subcanopy leaves. Reisolation studies confirmed that *Cercospora* had successfully become established on waterhyacinth and that much of the observed damage was due to *Cercospora*. The level of pathogen damage was low in April 1981, but increased significantly during the growing season. Mean values for pathogen damage in September 1981 were significantly higher than in October 1980. Reisolation of *Cercospora* in 1981 and the abundance of typical *Cercospora* symptoms confirmed that the fungus successfully overwintered and remained infectious on waterhyacinth. The increased level of pathogen damage

in 1981 suggested development of a *Ceratospora* population toward a level that could produce major impacts on the waterhyacinth population.

146. *Neochetina*. Based on the very low level of feeding by adult weevils during a site visit in March 1980, it was apparent that the *Neochetina* population was much lower than the population in other areas considered as study sites. This could have been due to either the relatively isolated waterhyacinth population at the study site or the routine treatment of most nearby waterhyacinth populations with herbicides, which effectively prevented development of a large *Neochetina* population in the general area. Although both adult and larval *Neochetina* increased during 1980, populations remained at relatively low levels compared to those in other areas. The higher population levels encountered in April 1981 than in October 1980 suggested that either winter conditions were not sufficiently severe to effect significant mortality of larval *Neochetina* or immigration of weevils from other areas occurred, and weevil reproduction in 1981 was well under way by April. The pronounced increase in adult weevils in July 1981 suggested a high survival rate of the first 1981 generation of *Neochetina*. The sharp increase in *Neochetina* larvae in July 1981 was due to a significant increase in adult weevils in April 1981. However, the number of adult and larval *Neochetina* decreased by September. This was unexpected because the waterhyacinth population during late summer consisted primarily of the large, Stage III plants normally preferred by *Neochetina*. A possible explanation was that the weevil population was sufficiently synchronized that the predominant life forms in September were eggs and first instar larvae. Gross inspection of plants would not have revealed the eggs, and many of the small first instar larvae would not have been found in internal waterhyacinth tissues. Although the number of adult weevils decreased in September 1981, the mean number of feeding scars increased significantly, which suggested higher levels of feeding by adults in September. However, feeding scars were recorded on a cumulative basis, and some of the feeding scars observed in September could have resulted from feeding by adults present in July. Although the *Neochetina* population increased significantly during the study, the observed decreases in waterhyacinth biomass and plant density probably resulted from the combined impacts of *Neochetina* and *Ceratospora* (see paragraph 149).

147. Sameodes. The failure of *Sameodes* to become established in the study area was evidenced by its absence on all sampling dates. Examination of the initial release site (1979) after 5 weeks confirmed that *Sameodes* had completed at least one life cycle. Empty pupal cases were found inside waterhyacinth petioles. However, no newly produced larvae or pupae were found. Since *Sameodes* adults are highly mobile, it was thought that the emerging moths might have immigrated to other nearby waterhyacinth populations. However, an intensive survey revealed no evidence of *Sameodes*. The failure of *Sameodes* to become established in the area was inexplicable. Waterhyacinths in the release area were of the Stage I morphotype, which is preferred. The release site was relatively sheltered by overhanging vegetation. Adults resulting from the original population would have had a large population of suitable plant material on which to oviposit, and relatively large numbers of adults should have emerged. Searches for *Sameodes* continued during 1979 and each sampling period in 1980 and 1981, but no individuals were found.

148. Other arthropods. Although both *Arzama* and *Orthogalumna* were found in plant samples, their occurrence was sporadic and they never occurred at sufficient population levels to significantly stress the waterhyacinth population.

149. Combined effects of *Cercospora* and *Neochetina*. The observed reduction in biomass and density of waterhyacinth was apparently due to the combined effects of *Cercospora* and *Neochetina*. *Cercospora* produces a phytotoxin, cercosporin, which produces a general necrosis of plant tissues and hastens senescence of waterhyacinth leaves. This decreases the total photosynthate produced by individual leaves, which results in a cumulative decrease in total primary production. Adult *Neochetina* feeding reduces the leaf surface available for photosynthesis; larval feeding interrupts normal flow of water and nutrients from leaves to rhizomes; and feeding activity of both adults and larvae produces large numbers of entry points available to weak, facultative plant pathogens. Although these species exerted insufficient stress to effect a reduction in percent cover of waterhyacinth, the combined activities of *Cercospora* and *Neochetina* resulted in decreased biomass and plant density. There was evidence that further reduction in biomass and density could be expected in the study area if the populations of *Cercospora* and *Neochetina* continued to expand.

Conclusions

150. Conclusions of this study were:

- a. The *Cercospora* formulation can be successfully applied with equipment normally used for large-scale pesticide applications.
- b. Sameodes failed to become established in the study area, but a combination of *Cercospora* and *Neochetina* effected a decrease in biomass and density of waterhyacinth. However, it was impossible to quantify the relative contribution of the two species to the observed reductions.

Neochetina and Spring Application of a Modified Cercospora Formulation

Purpose

151. The purpose of this study was to demonstrate the effectiveness of a combination of *Neochetina* and a spring application of a modified *Cercospora* formulation in controlling waterhyacinth in southern Louisiana.

Site selection and description

152. Site selection. Several potential study sites were evaluated in 1980 using the same criteria outlined in paragraph 129, with one addition: the selected site must already have a well-established *Neochetina* population of at least moderate population density.

153. Site description. The selected site (Figure 21) was a borrow pit (T14S, R10E) near Centerville in St. Martin Parish, which paralleled a bayou on one side and the Atchafalaya Basin levee on the other. A small berm separated the borrow pit from the bayou, and the only water connections to the bayou during normal or low flow periods were three narrow channels across the berm. Although water from the bayou flowed through the borrow pit during peak flow periods, dense emergent vegetation along the berm effectively prevented waterhyacinths from being transported out of the study area. The borrow pit was completely covered by a uniform-sized waterhyacinth population, and a site visit revealed moderate to intense feeding by adult *Neochetina*. Although waterhyacinth populations in the bayou were routinely controlled by herbicide applications, there was no evidence that the waterhyacinth population in the borrow pit had been sprayed in recent years.

Materials and methods

154. Establishment of study area. A 6.4-acre portion of the borrow pit



Figure 21. Centerville study site immediately prior to application of *Cercospora* in 1981

was selected, and barriers (paragraph 130) were placed across each end of the study area. The study area was 0.45-mile in length and averaged 117 ft in width.

155. *Cercospora* formulation. Because the original *Cercospora* formulation had a short shelf-life, consisted of highly variable particle sizes, and contained considerable amounts of contaminants, Abbott Laboratories modified the formulation to produce a more acceptable commercial formulation. The modified formulation was a fluffy white powder containing thick-walled vegetative cells. The formulation had a longer shelf-life (6 months), more uniform and smaller particle sizes, and fewer contaminants than the original formulation. The viability of *Cercospora* in the modified formulation was 1.0×10^6 CFU/g, which was nearly twice that of the original formulation.

156. Application of *Cercospora*. The *Cercospora* formulation was applied by fixed-wing aircraft at 1600 hr on 22 April 1981 at a rate of 1 lb of formulation per acre (2.0×10^5 CFU/m²). The formulation was suspended in 247 gal of tap water, and 1 pt of Ortho X-77 was added as a surfactant. The application system was identical to the system employed in the application at Lake Theriot (paragraph 133). The pilot made nine passes over the study area

at an average height of 10 ft above the waterhyacinth canopy. The application resulted in total wetting of the waterhyacinths, and the formulation particles readily adhered to the leaves. Winds were calm, the sky was overcast, and the ambient temperature was 82°F.

157. Sampling procedure. The study site was divided into five sections of equal size and two sampling points in each section were randomly selected. Three quadrats (0.5 m 0.5 m) were sampled at each point. The same procedure was employed for characterizing plant and animal populations in this study as described for the Lake Theriot study (paragraphs 134-137). Pretreatment data were collected in August 1980 and April 1981, and posttreatment data were collected in July and September of 1981. The study area was sampled in August 1980 because the site had originally been selected to receive a fall application of *Cercospora*, but sufficient quantities of formulation were not available at that time.

158. Data analysis. The same analytical procedures were employed for this study as described for the Lake Theriot study (paragraphs 138-140).

Results

159. Waterhyacinth population. Percent cover of waterhyacinth in the study area remained at 100 percent throughout the study (Table 5). Plant density, height, and daughter plant production were typical of the normal pattern for waterhyacinth growth in southern Louisiana. Although biomass values followed the typical pattern for waterhyacinth growth, the mean biomass was 21.5 kg/m² in August 1980 and 17.3 kg/m² in September 1981, a significant reduction of approximately 20 percent.

160. Pathogen damage. The mean pathogen damage value (Figure 22) was moderate (2.67) in August 1980, decreased significantly to 1.89 in July 1981, and then increased significantly to a maximum of 3.12 in September 1981. The mean value for September 1981 was significantly greater than the mean value for August 1980. Efforts to reisolate *Cercospora* from waterhyacinth on both posttreatment sampling dates were unsuccessful. Samples of the *Cercospora* formulation were also applied to waterhyacinth plants under laboratory conditions, but the plants did not become infected.

161. Arthropod species. Although the mean number of *Neochetina* adults (Figure 23) decreased slightly from 38.8/m² in August 1980 to 25.6/m² in April 1981 and remained at approximately that level until September 1981, the

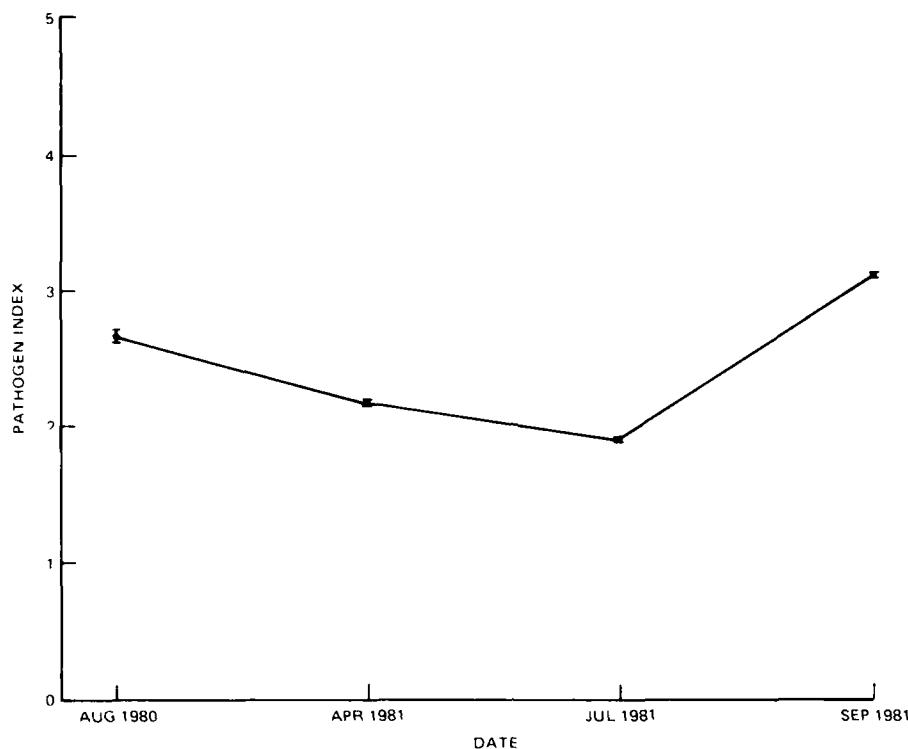


Figure 22. Mean pathogen damage index values/leaf for Centerville study site. Vertical bars represent two standard errors of means

changes were not significant. The mean index value for adult *Neochetina* feeding (Figure 24) decreased significantly from 2.14 in August 1980 to a minimum of 1.21 in July 1981, and then increased significantly to a maximum of 2.96 in September 1981. The mean number of *Neochetina* larvae (Figure 23) increased significantly from 68.9/m² in August 1980 to 185.1/m² in July 1981, and then declined significantly to 54.1/m² in September 1981, which was approximately equal to larval density in August 1980. No *Sameodes* or *Orthogalumna* were found on the site, and the population density of *Arzama* was very low (Table 6).

Discussion

162. Waterhyacinth population. Seasonal variation in mean values for all examined parameters were typical of waterhyacinth populations in southern Louisiana. Plant densities and daughter plant production were highest in early spring and lowest during late summer. Biomass and plant heights were highest during late summer and declined significantly during the winter months

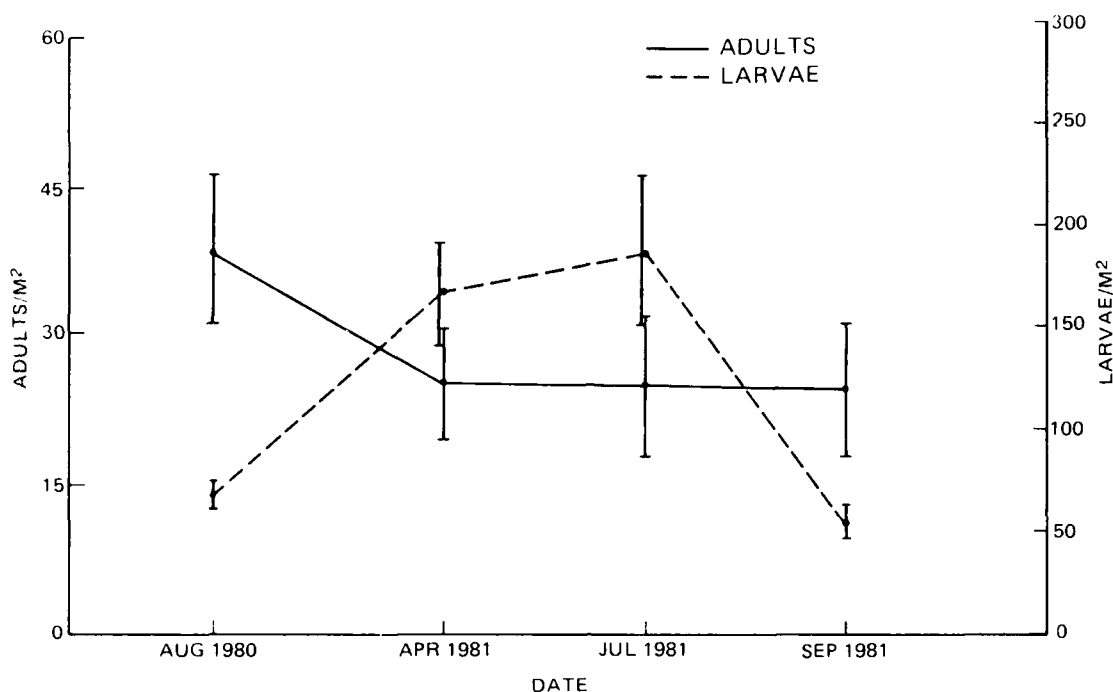


Figure 23. Mean numbers of *Neochetina* adults and larvae/m² at the Centerville study site. Vertical bars represent two standard errors of means

to their lowest levels in early spring. However, biomass values were 20 percent lower in September 1981 than in August 1980. Although this difference could have been due to annual fluctuations in waterhyacinth growth as a result of slight changes in weather patterns, one or more biological agents probably contributed significantly to the change. The reduction in biomass was not due to either herbicide applications or dewatering.

163. Pathogen damage. Although mean pathogen damage increased significantly in September 1981 as compared to August 1980, the increase was not pronounced. This suggested that *Cercospora* did not reach a sufficient population level to impact the waterhyacinth population. Coupled with the fact that *Cercospora* could only rarely be reisolated from the study area, these data indicated that *Cercospora* did not become established. However, viability tests performed on the inoculum immediately prior to application yielded a *Cercospora* viability of 1×10^6 CFU/g of formulation. Failure of *Cercospora* to become established on either laboratory or field plants strongly suggested that *Cercospora* in the formulation lacked sufficient virulence to achieve infection. The apparent lack of virulence was due either to low virulence of

- a. St. Charles Parish. Abundant *Sameodes* populations were found 10 km east and south of the Cypress Canal release site. Both larvae and pupae were found at four locations in this area and population development was sufficient to produce visual impacts on the plant population at two locations. Additional *Sameodes* larvae and pupae were also found at scattered locations between the four areas identified in Table 10.
- b. Jefferson Parish. Sparse populations of *Sameodes* were observed in a canal that paralleled US Highway 90 in Jefferson Parish (west of the Mississippi River), which represented the eastern limits of *Sameodes* distribution in Louisiana in 1980.
- c. Lafourche Parish. Abundant *Sameodes* larvae and pupae were found in a canal that paralleled US Highway 90, 6.6 km east of its junction with Louisiana Highway 316 in Lafourche Parish. However, the population had not developed sufficiently to significantly impact the waterhyacinth population.
- d. Terrebonne Parish. A large population of *Sameodes* was found in a canal that paralleled US Highway 90 at its junction with Louisiana Highway 24, approximately 11.5 km east of Houma. This site, which was 24 km northeast of the Lake Theriot release site and 73 km west of the Cypress Canal release site, represented the western limits of known *Sameodes* distribution in Louisiana in 1980. The location at which APML personnel found *Sameodes* within the Houma city limits in October had been sprayed with herbicides, and no waterhyacinths were present.
- e. St. John the Baptist Parish. Four additional *Sameodes* adults were collected by Mr. Brou at Edgard during 1980 (Table 9). Although these individuals were not found on a waterhyacinth population, the collections represented the northern limits of known *Sameodes* distribution in 1980.

196. Winter survey in January 1981. To determine the effects of freezing temperatures on the *Sameodes* population in Louisiana, a January 1981 survey was conducted of all sites where *Sameodes* had been found during November 1980. Freezing temperatures (minimum of -7°C) occurred on most nights during late December 1980 and the first 2 weeks of January 1981. Although only two *Sameodes* larvae (third and fifth instar) were found at Cypress Canal (St. Charles Parish), they were active when the water temperature was 8°C and the ambient temperature was -7°C .

197. 1981 surveys. Based on routine inspections of sites where *Sameodes* had been found in 1980 and a survey conducted in October 1981, *Sameodes* was found to be more widely distributed in 1981 than in 1980 (Table 11). *Sameodes* was found in most areas where it had occurred in 1980, and was also found farther west, north, and east than in 1980. The following summarizes 1981 observations:

pupae were provided to LDWF waterhyacinth control personnel, who routinely inspected waterhyacinth populations in their areas and reported any observations of *Sameodes* to Mr. Manning. Follow-up site visits were made by WES personnel to confirm the presence of *Sameodes*.

192. While on a field mission in Louisiana in October 1980, Dr. Ted Center and Mr. Wiley Durden of the APML examined waterhyacinth populations in the area between Houma and New Orleans for the presence of *Sameodes*.

193. Mr. Vernon Brou, an expert on Lepidoptera of Louisiana, routinely collects insects in a light trap at his home in Edgard (St. John the Baptist Parish). Since his home was only 17 km from the Cypress Canal release site, Mr. Brou was asked to provide any records of *Sameodes* collected in his light trap during 1980 and 1981.

Results

194. Although *Sameodes* was not found at either the Lake Theriot release site or study area in 1980 or 1981, there was evidence that at least a few adults emerged from the released individuals (paragraph 147). The original population released at Cypress Canal resulted in a few individuals being found in October 1979 near the release site, but no significant population developed. These findings led to additional releases at both sites in June 1980. It was learned in August 1980 that Mr. Vernon Brou had captured an adult *Sameodes* on 30 May in a light trap at his home. Since this collection was made prior to the 1980 releases, it provided evidence that *Sameodes* had become established in the area in 1979 and had successfully overwintered. Additional collections of *Sameodes* by Mr. Brou in 1980 and 1981 are presented in Table 9. A survey by Dr. Ted Center and Mr. Wiley Durden (USDA-APML) in October 1980 revealed *Sameodes* larvae and pupae at two sites on the northern end of Cypress Canal and in canals at two locations along US Highway 90 in Jefferson Parish. They also found a *Sameodes* pupa in Bayou Terrebonne within the Houma city limits (Terrebonne Parish), approximately 78 km west of Cypress Canal and 15 km northeast of Lake Theriot.

195. WES Survey in November 1980. Since *Sameodes* had become established in a fairly extensive area west of New Orleans during 1980, WES personnel conducted a survey for *Sameodes* in November 1980. Locations of *Sameodes* occurrence are presented in Table 10, and observations are presented in the following subparagraphs:

Stage III morphotype, which would not be conducive to development of a *Sameodes* population.

- d. Pecan Island. The selected release site (T15S, R1E) was a canal located 11.5 km east of Pecan Island in Vermilion Parish. The canal extended in a southerly direction from Louisiana Highway 82, and a gravel road paralleled the west bank. The canal was approximately 3.5 km in length and averaged 18 m in width. An extensive waterhyacinth mat composed of predominantly Stage I plants was present, and the entire water surface in some areas was covered by waterhyacinths. The waterhyacinth mat extended only 3 to 4 m from the shore in other areas, leaving the central portion of the canal available for expansion of the plant population. The release site was not delimited by barriers.

188. Release of *Sameodes*. Methods used for the *Sameodes* releases at Lake Theriot and Cypress Canal were described in paragraphs 132 and 171, respectively. The release at Grand Lake was effected in June 1981 by placement of 5000 eggs and first instar larvae produced at WES on waterhyacinths using the method described by Center (1981a). The release at Pecan Island was made in August 1981 by placing *Sameodes*-infested plants from WES greenhouses among the waterhyacinth population in the canal. Approximately 1000 individuals of various life stages were released.

189. Survey methods. Data on the establishment and distribution of *Sameodes* in Louisiana were obtained from four sources: WES surveys, LDWF, USDA-APML personnel, and private individuals.

190. Personnel from WES conducted routine surveys at the two 1979 release sites throughout the study, including a winter survey in January 1981. Intensive surveys were also conducted throughout southern Louisiana in November 1980 and October 1981. After learning in 1980 that *Sameodes* had become established, a radial survey method was employed in which waterhyacinth populations were examined in all cardinal directions from the release sites. When new *Sameodes* populations were found, the radial survey method was again employed using the newly found locations as focal points. *Sameodes* locations were carefully recorded and these sites were included on all subsequent surveys. No attempt was made to quantify the *Sameodes* population at any site, but relative descriptors (e.g. abundant, moderate, sparse) were used to indicate the degree of population development and damage produced by *Sameodes*.

191. Mr. James Manning of the LDWF assisted in the November 1980, January 1981, and October 1981 surveys. Specimens of *Sameodes* larvae and

- b. Although both adult and larval *Neochetina* contributed to the observed decline, larvae produced greater impacts on the plant population.
- c. Greater levels of pathogen damage in July 1981 than in July 1980 could have contributed to the decline in waterhyacinth during 1981, but pathogen damage alone did not account for the magnitude of the decline.
- d. *Sameodes* did not become established in the study area, and did not contribute to the observed decline in the plant population.

Establishment, Dispersal, and Distribution of *Sameodes*

Purpose

185. The purpose of this study was to establish *Sameodes* on waterhyacinth in southern Louisiana, and to monitor its dispersal and distribution in the state.

Selection and description of release sites

186. Site selection. Original sites selected for the release of *Sameodes* were at Lake Theriot (paragraph 129) and Cypress Canal (paragraph 168). Two additional release sites were selected in 1981 using the following criteria:

- a. Presence of small, bulbous-petioled (Stage I) waterhyacinths.
- b. Fringe growth of waterhyacinth with ample area for population expansion.
- c. Unlikelihood of herbicide spraying.
- d. Locations within the Atchafalaya Basin and west of Lafayette (one each).

187. Site descriptions. The following sites were selected for *Sameodes* releases:

- a. Lake Theriot. See paragraph 129.
- b. Cypress Canal. See paragraph 168.
- c. Grand Lake. The release site (T14S, R10E) was located in a backwater area north of Gray Horse Island near a boat launch on the west side of Grand Lake in St. Martin Parish. The site was adjacent to the levee on the west side of the Atchafalaya Basin. A waterhyacinth mat consisting of Stage I plants extended for a distance of 5 to 7 m from the shore, and there was ample open water for continued expansion of the plant population. No effort was made to restrict movement of waterhyacinths out of the release site because such an effort would increase the likelihood of the plants to convert to the

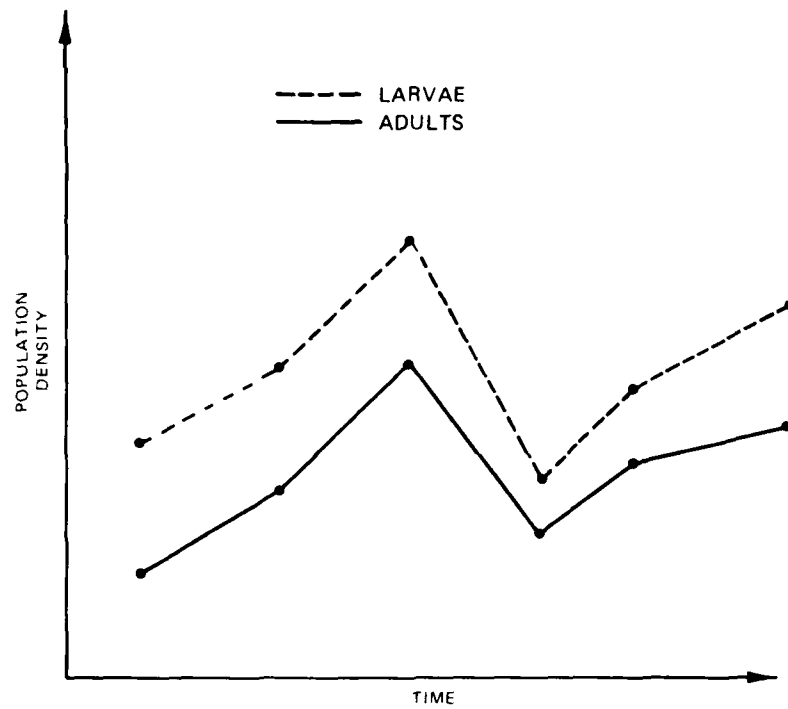


Figure 30. Generalized asynchronous pattern of population development

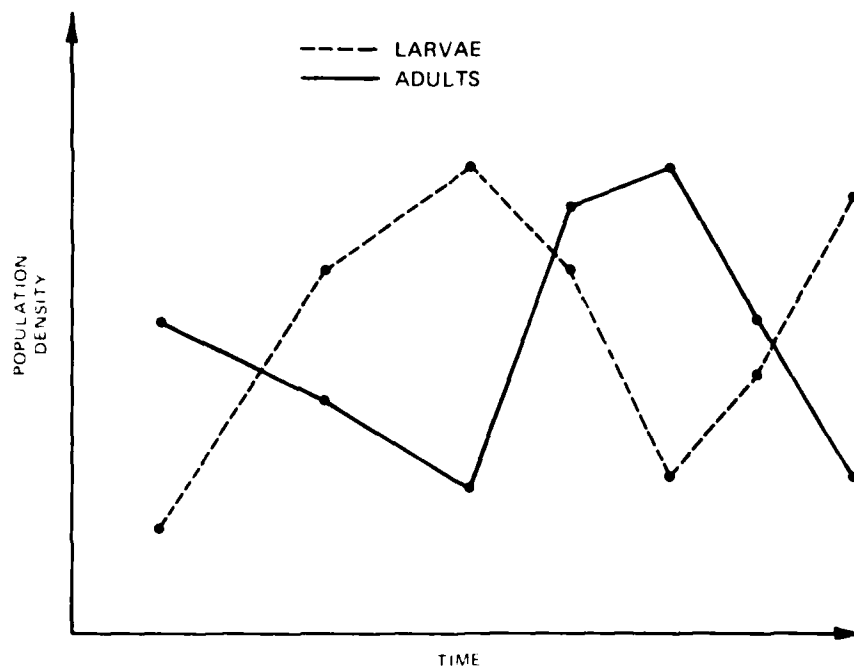


Figure 31. Generalized synchronous pattern of population development

182. Arthropod species. Only *Neochetina* occurred in sufficient numbers to impact the waterhyacinth population. The population dynamics of *Neochetina* were generally typical of the expected pattern, in which maximum populations of both adults and larvae were greater during mid to late summer than during the spring. However, the population appeared to be asynchronous (Figure 30), in which the numbers of adults and larvae varied in a similar manner among sampling dates. The mean number of larvae per plant exceeded the mean number of adults on all sampling dates. Whether an asynchronous population is more desirable than a synchronous population is debatable. A synchronous population (Figure 31) can lead to large numbers of larvae followed by a large population of adults, while an asynchronous population results in significant numbers of both adults and larvae at all times, thus placing maximum, continued stress on the plant population. However, an asynchronous population has a higher degree of stability, and is less likely to decline significantly due to external factors than a synchronous population. The asynchronous *Neochetina* population in this study effected continual stress on waterhyacinth throughout the 1981 growing season due to feeding by both adults and larvae. However, increased larval feeding appeared to produce the major impact on the plant population, especially during July and September of 1981. The *Neochetina* population appeared to be expanding in 1981, as evidenced by the higher larval population in July and September of 1981 than for corresponding periods in 1980. The failure of *Sameodes* to become established was probably related to the predominantly Stage III plants in the study area, which are less preferred as oviposition sites by *Sameodes*. Although the Stage I plants normally preferred by *Sameodes* were present during April 1981, the waterhyacinth population quickly reverted to the Stage III morphotype. *Arzama* and *Orthogalumna* populations were sporadic, and did not occur at sufficient levels to significantly impact the waterhyacinth population.

183. Combination of *Neochetina* and *Sameodes*. Since *Sameodes* failed to become established in the study area, no combined effects of these species on the waterhyacinth population were observed.

Conclusions

184. Conclusions of this study were:

- a. A 40-percent reduction in plant cover and a decreased waterhyacinth biomass were attributed primarily to an expanding *Neochetina* population.

179. Mean numbers of *Aureocella* larvae/m² remained at low levels throughout the study, never reaching levels of 1.0 larvae/m² (Table 8). Mean index values for *Orthogalium* tunnels increased in 1981, but remained at low levels throughout the study (Table 8).

Discussion

180. Waterhyacinth population. The waterhyacinth population in 1980 generally exhibited a growth pattern typical for the species in southern Louisiana. Plants were initially small and numerous, but biomass increased and plant density decreased by fall. However, the waterhyacinth population did not exhibit the typical pattern during 1981. Mean biomass in September 1981 had not developed to levels achieved in 1980, and there was a 40-percent reduction in plant cover. These findings strongly suggested that one or more environmental factors were significantly impacting the waterhyacinth population. The area had received no herbicide applications and was not dewatered. Although alligatorweed interspersed among the waterhyacinth plants assumed aspect dominance during the early spring of both 1980 and 1981, interspecific competition was ruled out as a possible explanation for the reduction in percent cover and biomass of waterhyacinth because *Agasicles hygrophila* (Selmon and Vogt) virtually eliminated alligatorweed by June of both years. There were no significant variations in the weather pattern. By eliminating the above factors as possible explanations for the observed decrease in percent cover and biomass of waterhyacinth in 1981, it became evident that one or more biological agents were probably responsible for the observed changes in the waterhyacinth population.

181. Pathogen damage. Although pathogen damage remained relatively constant except for significantly lower values in July 1980, it is possible that pathogen damage contributed to the observed decrease in waterhyacinth biomass and percent cover in 1981. Pathogen damage was much greater in July 1981 than in July 1980, and remained relatively high during 1981. However, no strongly virulent plant pathogens were isolated from the study area, and it is probable that the higher level of pathogen damage in July 1981 resulted from increased activity by weak, facultative pathogens as the waterhyacinth population was subjected to other stress factors. Pathogen damage was greatest on older, rapidly senescing waterhyacinth leaves and petioles.

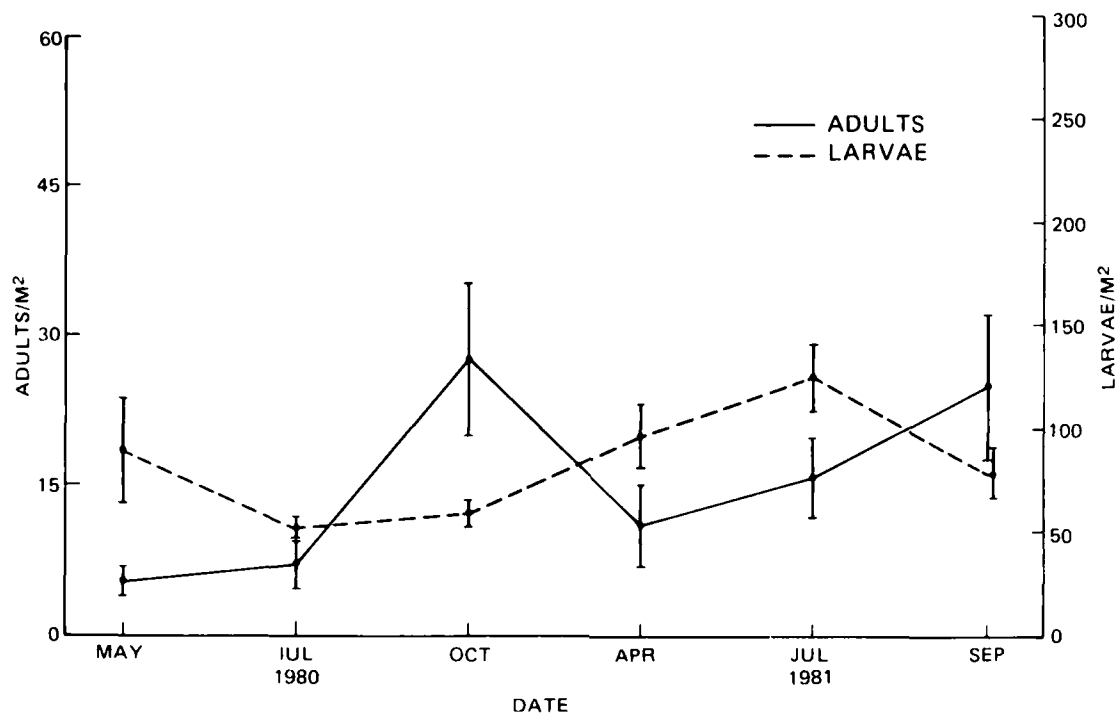


Figure 28. Mean numbers of *Neochetina* adults and larvae/m² at the Cypress Canal study site. Vertical bars represent two standard errors of means

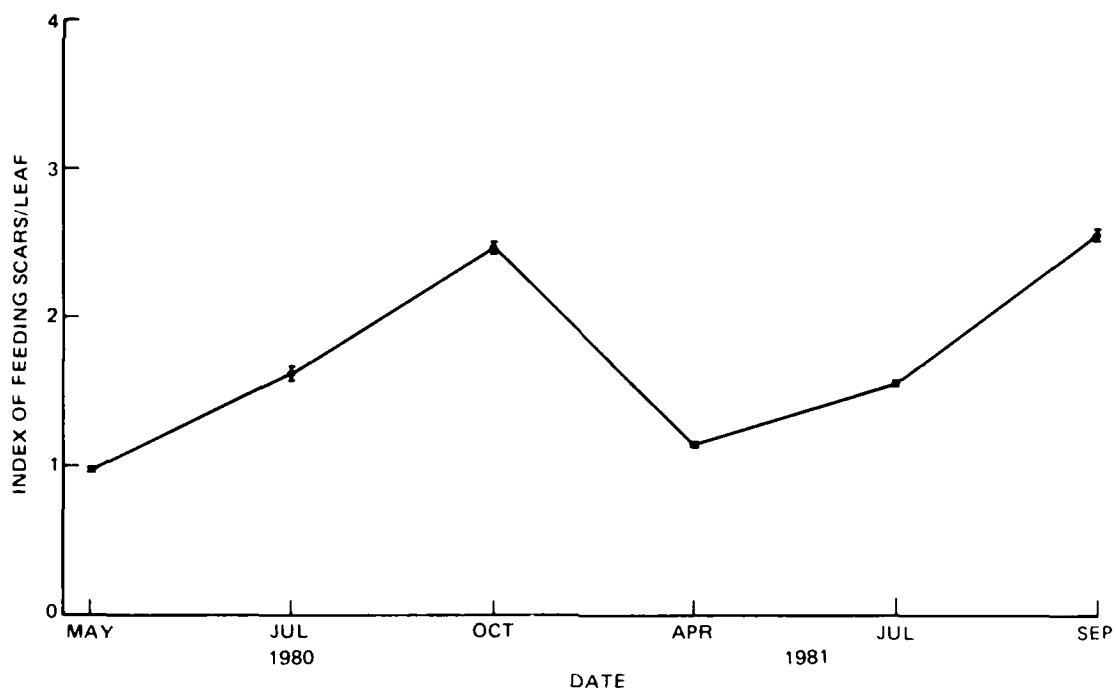


Figure 29. Mean values of feeding scars/leaf by adult *Neochetina* at the Cypress Canal study site. Vertical bars represent two standard errors of means

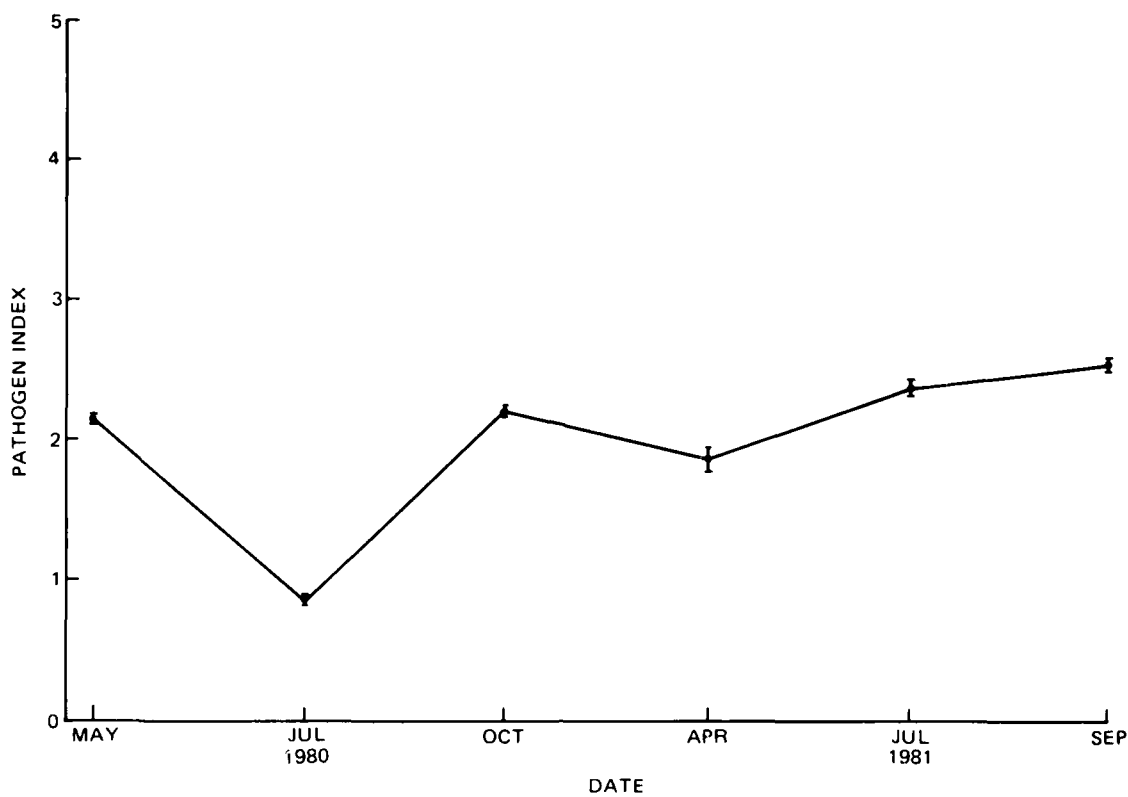


Figure 27. Mean pathogen damage index values/leaf for Cypress Canal study site. Vertical bars represent two standard errors of means

number of *Neochetina* larvae/m² (Figure 28) decreased significantly from 88.9/m² in May 1980 to 50.1/m² in July 1980, and decreased significantly from 124.8/m² in July 1981 to 78.6/m² in September 1981. The mean number of larvae was significantly higher in July 1981 than in July 1980.

178. No larvae or new pupae were found at the release site 5 weeks after the initial *Sameodes* release. Although one moth resembling *Sameodes* was observed, efforts to capture it failed. Both adults and larvae were found at the release site in October 1979. However, *Sameodes* was not found at either the release site or study area in 1980. *Sameodes* larvae were found in a portion of Cypress Canal approximately 0.5 mile north of the study area in October 1980 by Dr. Ted Center and Mr. Wiley Durden of the APML. This finding, together with other observations detailed in paragraphs 189-197 confirmed that *Sameodes* was established on waterhyacinth in the general area, but *Sameodes* was not found in the study area in 1981.

Results

174. Waterhyacinth population. Percent cover of waterhyacinth in the study area remained at 100 percent for all 1980 sampling dates, but decreased to 60 percent in September 1981 (Table 7). Mean biomass (weighted by percent cover) was 7.8 kg/m^2 in May and July of 1980, increased to a maximum of 10.9 kg/m^2 in October 1980, and decreased to 6.8 kg/m^2 or less for all 1981 sampling dates (Table 7). Mean biomass values (weighted by percent cover) were lower in July and September of 1981 than for corresponding dates in 1980. Mean plant density (weighted by percent cover) exceeded $100/\text{m}^2$ in both May 1980 and April 1981, and declined to $28.5/\text{m}^2$ in October 1980 and $15.1/\text{m}^2$ in September 1981 (Table 7). Mean plant height increased significantly from 23.1 cm in May 1980 to 56.3 cm in October 1980, decreased to 21.1 cm in April 1981, and then increased significantly to a maximum of 65.3 cm in September 1981 (Table 7). The mean number of daughter plants was approximately $13.4/\text{m}^2$ in May and July of 1980, decreased significantly to $2.3/\text{m}^2$ in October 1980, increased significantly to a maximum of $60.7/\text{m}^2$ in April 1981, and then decreased significantly to approximately $12.5/\text{m}^2$ in July and September of 1981 (Table 7). The mean value for April 1981 was significantly higher than for May 1980, and the mean for October 1980 was significantly lower than for September 1981.

175. Pathogen damage. Mean index values of pathogen damage (Figure 27) ranged from a low of 0.86 in July 1980 to a maximum of 2.49 in September 1981. The mean value for July 1980 was significantly lower than for all other sampling dates.

176. Arthropod species. Results for *Neochetina*, *Sameodes*, and *Arzama* and *Orthogalumna* are discussed in the following paragraphs.

177. The mean number of *Neochetina* adults/ m^2 (Figure 28) increased significantly from $5.7/\text{m}^2$ in May 1980 to $27.6/\text{m}^2$ in October 1980, decreased significantly to $11.2/\text{m}^2$ in April 1981, and then significantly increased to a maximum of $25.2/\text{m}^2$ in September 1981. Means for *Neochetina* adults were significantly higher in July 1981 than in July 1980. The mean values for *Neochetina* feeding scars per leaf (Figure 29) increased significantly from 0.98 in April 1980 to a 1980 maximum of 2.47 in October, and from 1.13 in April 1981 to a 1981 maximum of 2.55 in September. Means for *Neochetina* feeding scars were significantly higher in April 1981 than in May 1980. The mean

greenhouses and was transported to the release site within or on waterhyacinth plants. The release was effected by placing *Sameodes*-infested plants among waterhyacinths present at the site (Figure 26). Examination of the *Sameodes* colony immediately prior to the release revealed only two dead larvae, both of which apparently drowned in the tubs of water used for transporting the



Figure 26. Release of *Sameodes* at the Cypress Canal study site in May 1979

infested plants. An additional 800 eggs and first instar larvae were released in June 1980. Individuals for this release were produced at WES by the method described by Center (1981a).

172. Sampling procedure. The same sampling procedure was employed for this study as described for the Lake Theriot study (paragraphs 134-137). Since it was anticipated that several generations would be required for the *Sameodes* population to develop to detectable levels in the study area, a decision was made to begin routine sampling in May 1980, the data from which were to be considered as pretreatment data. Subsequent sampling was conducted in July and October of 1980, and in April, July, and September of 1981.

173. Data analysis. The same analytical procedures were employed for this study as described for the Lake Theriot study (paragraphs 138-140).

169. Site description. The selected site (Figure 25) was Cypress Canal (T14S, R21E), located 3.2 km south of Boutte in St. Charles Parish. The canal extended southeastward to Lake Salvador, and water flow was from a northwesterly to southeasterly direction. Water depth ranged from 2 m to 4 m, and the water surface was completely covered by waterhyacinth. A gravel road paralleled the canal on the east side and a cypress-tupelo swamp bordered the canal on the west side.



Figure 25. Cypress Canal study site in May 1980

Materials and methods

170. Establishment of study area. The study area consisted of a 1.45-km portion of the canal, which averaged 15 m in width. Barriers (paragraph 130) were placed across the canal at each end of the study area to retain the plant population. A site visit in April 1979 revealed significant feeding of *Neochetina* adults.

171. Release of *Sameodes*. A site located approximately 100 m south of the study area was selected for the *Sameodes* release. The release site was sheltered by overhanging vegetation and consisted of predominantly Stage II plants. An estimated 5000 *Sameodes* eggs, larvae (all instars), and pupae were released in May 1979. The colony used for the release was produced in WES

Neochetina adult feeding was significantly greater in September 1981 than in August 1980, even though the mean number of adults per plant was slightly less in September 1981. Whether this difference was due to changes in the nutrient content of waterhyacinth leaves from August to September or to physiological changes in the adult weevils in response to reduced photoperiods is not known. The lower waterhyacinth biomass in September 1981 than in August 1980 could have resulted from intensive *Neochetina* larval feeding during July 1981, followed by stable numbers of adults during August and September of 1981. The population of *Arzama* was so low that it exerted little, if any, pressure on the waterhyacinth population.

165. Combination of *Cercospora* and *Neochetina*. Because *Cercospora* failed to become established, there was no combined effect of *Cercospora* and *Neochetina* on waterhyacinth.

Conclusions

166. Conclusions of this study were:

- a. Although the modified *Cercospora* formulation was more suitable for application and had a higher concentration of viable particles than the original formulation, the propagules lacked sufficient virulence to infect the treated plants; therefore, *Cercospora* had no effect on the waterhyacinth population.
- b. The observed decrease in waterhyacinth biomass was probably due to an increase in the population of *Neochetina* during the 1981 growing season, with the principal effects being due to larval feeding early in the growing season and adult feeding later in the season.

Neochetina and *Sameodes*

Purpose

167. The purpose of this study was to demonstrate the effectiveness of a combination of *Neochetina* and *Sameodes* in controlling waterhyacinth in southern Louisiana.

Site selection and description

168. Site selection. Several potential study sites were evaluated during 1979 using the criteria listed in paragraph 129. In addition, the selected site was required to have a moderate to dense population of *Neochetina*.

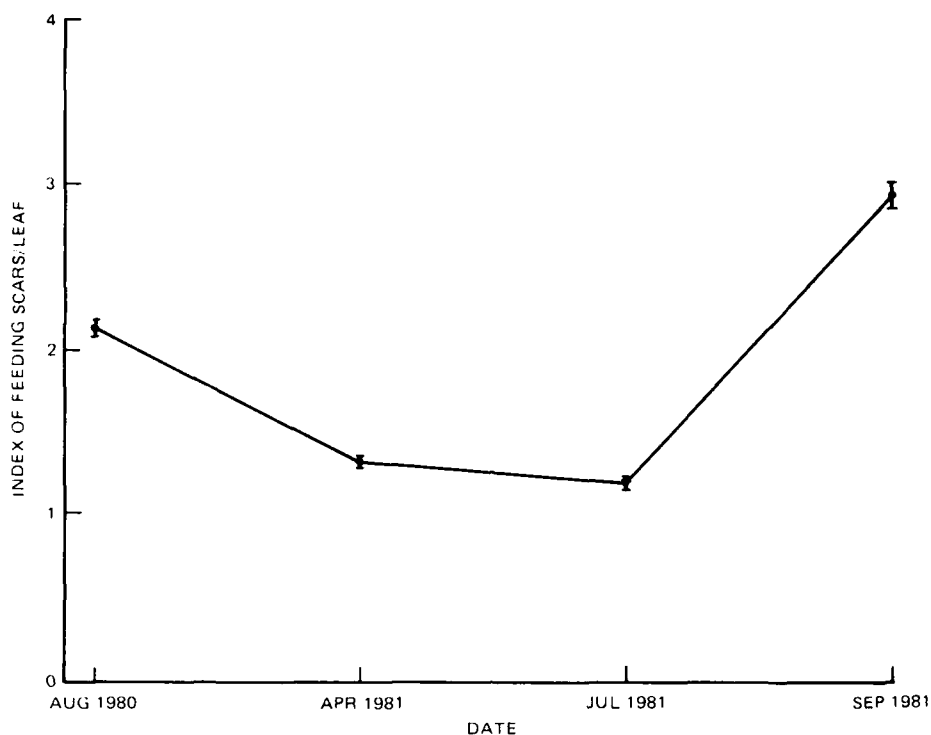


Figure 24. Mean values of feeding scars/leaf by adult *Neochetina* at the Centerville study site. Vertical bars represent two standard errors of means

stock cultures or to the manner in which the formulation was produced. Regardless, the effort to establish *Cercospora* on waterhyacinths in the study area was unsuccessful, and the observed level of pathogen damage was attributed to an endemic group of weak, facultative pathogens and saprophytes.

164. Arthropods. Moderate populations of *Neochetina* were present on all sampling dates. However, the abundances of larvae and adults were inversely correlated on all sampling dates, with peaks in adults occurring in August 1980 and September 1981 when larval populations were relatively low. The significant increase in larvae in April 1981 probably resulted from a combination of increased numbers of overwintering larvae produced late in 1980 and larvae resulting in 1981 from oviposition by overwintering adults. Three months of the 1980 growing season remained after 1 August for population development, and oviposition by adults in 1981 could have begun as early as 1 March. The relationship of adult and larval populations in September 1981 and August 1980 was similar, and mean numbers of both life stages were similar, which suggested a relatively stable *Neochetina* population. However, the level of

- a. St. John the Baptist Parish. Abundant *Sameodes* were found in a canal at the intersection of Interstate-55 and Interstate-10, 3 km east of LaPlace, which represented the first observation of *Sameodes* east of the Mississippi River in Louisiana. The population was producing visible impacts on the waterhyacinth population. All larval instars and pupae were found, including numerous individuals on Stage III plants. Moderate populations of *Sameodes* were also found 3.2 km north of the intersection in canals paralleling Interstate-10.
- b. Jefferson Parish. A small population of *Sameodes* was found in a canal paralleling US Highway 90, located 16.1 km west of Westwego. This was one of two locations in which *Sameodes* had been found in Jefferson Parish in 1980.
- c. Lafourche Parish. Abundant larvae and pupae were found at a boat launch on the east side of Bayou Des Allemands where US Highway 90 intersects the bayou. Infested plants had drifted into the area from the north, which suggested that *Sameodes* populations were present in the Lake Des Allemands area. Fifth instar larvae and pupae were found on Stage III plants at this location.
- d. Terrebonne Parish. *Sameodes* were found at six locations in Terrebonne Parish. A large population was present in a canal 0.8 km east of Houma, and the waterhyacinth population was severely stressed. Abundant larvae were found at two locations in Bayou Terrebonne within the Houma city limits. Sparse populations of *Sameodes* were also found south of Houma in a canal that paralleled Louisiana Highway 315, and at two locations in Bayou Black (4.9 km and 8.0 km west of Houma). These observations represented the southern and western limits of known *Sameodes* distribution in Louisiana in 1981.
- e. St. Charles Parish. *Sameodes* was found at six locations in St. Charles Parish. Dense populations of larvae and pupae were found in Sellers Canal and in a canal at Paradis. A sparse population was also found in a canal that paralleled US Highway 61 at Norco.
- f. St. James Parish. Abundant *Sameodes* were found in a 3.2-km portion of a canal paralleling US Highway 61 near Gramercy.
- g. Ascension Parish. Numerous *Sameodes* larvae were found in several canals near Sorrento, which represented the known northern limits of *Sameodes* distribution in Louisiana in 1981.
- h. Light trapping of *Sameodes*. Mr. Brou collected a total of 16 adult *Sameodes* (Table 9) in a light trap at Edgard from July to November 1981.

198. Observations at 1981 *Sameodes* release sites. No evidence was found in October that *Sameodes* had become established at either the Grand Lake (St. Martin Parish) or Pecan Island (Vermilion Parish) release sites.

Discussion

199. Although there was evidence that *Sameodes* had survived at the Cypress Canal release site in 1979, the failure to observe significant populations during the 1979 growing season led to speculation that *Sameodes* had not become established. However, the adult *Sameodes* captured by Mr. Brou in May 1980 confirmed that *Sameodes* not only became established in 1979, but also successfully overwintered. Apparently, adults emerging from the release site emigrated to other waterhyacinth populations that were more suitable as oviposition sites. This same pattern of establishment was noted by Center (1981a) for *Sameodes* populations in Florida. The *Sameodes* adult collected by Mr. Brou also confirmed that *Sameodes* had dispersed at least 17 km northwest of the Cypress Canal release site during 1979 and early 1980. The failure to find *Sameodes* populations in the Lake Theriot area during 1979 and 1980 suggested that the species had failed to become established. However, the extensive surrounding marsh contained large populations of waterhyacinth, and it is possible that adults emerging from the released colony moved out of the release area and became established on other waterhyacinth populations.

200. Surveys in October and November 1980 revealed that *Sameodes* had not only become established in the Cypress Canal area, but also had become fairly widely distributed. By November 1980, *Sameodes* occurred in an area encompassing 1230 km², including all or portions of five parishes. Dispersal appeared to be primarily in a westerly direction from the Cypress Canal release site. However, *Sameodes* had not become established east of the Mississippi River, and there was concern that the river might serve as a natural barrier to limit eastward dispersal. The wide distribution of *Sameodes* in southern Louisiana and occurrence of abundant populations in some areas by November 1980 increased the likelihood of it successfully overwintering in 1981. Populations occurred in a variety of site conditions, ranging from open canals and marshes to canals and swamps sheltered by overhanging vegetation. The discovery of active larvae in January 1981 after an extended period of freezing temperature indicated that *Sameodes* can tolerate the winter environment of Louisiana.

201. *Sameodes* was not found during the early part of the 1981 growing season. No evidence could be found as late as June that *Sameodes* had overwintered at any site where it had occurred in 1980. This was surprising, since

active larvae were found in January 1981. Although freezing temperatures probably resulted in the death of many individuals, the greatest impact of freezing temperatures on *Sameodes* was probably the partial destruction of small, bulbous-petioled plants in which they overwintered. As leaves and petioles of these plants were destroyed by freezing, plant buoyancy decreased and the plants floated lower in the water. Increased waterlogging of the remaining petioles probably resulted in drowning of numerous larvae and pupae. In areas where this occurred, survival was probably limited to those individuals that were present in the larger Stage II and Stage III plants. Some individuals may also have survived in Stage I plants in sheltered areas, especially those having a southern or eastern exposure.

202. *Sameodes* was first found in 1981 on July 20 when Mr. Brou captured an adult in his light trap. The first field evidence of *Sameodes* in 1981 was found in August in a canal intersecting US Highway 90 in St. Charles Parish. The failure of *Sameodes* to develop to detectable population levels until July in 1981 was attributed to significant reductions in the population during the previous winter. This caused concern because the greatest potential for *Sameodes* to impact waterhyacinths in Louisiana is during early spring when most waterhyacinth populations consist predominantly of Stage I plants. Most waterhyacinth populations convert to the Stage III morphotype by July, and the Stage III morphotype is not as susceptible to infestation by *Sameodes*.

203. *Sameodes* population development was rapid during August and September 1981, and abundant populations occurred at several locations in October, including a site on the east side of the Mississippi River near LaPlace (St. John the Baptist Parish). This confirmed that *Sameodes* had successfully bridged the potential natural barrier of the river. The 1981 distribution had exceeded the 1980 distribution by October, especially in a northerly direction. *Sameodes* occurred 30 km farther north in 1981 than in 1980, and the total 1981 range covered 2883 km² in all or portions of nine parishes. However, westward and southern expansion of the range was limited during 1981. The only significant increase from the 1980 distribution was 25 km farther south of Houma and 15 km farther west of Houma. Factors limiting the southern and westward dispersal of *Sameodes* are not known. There were no perceptible changes in climatic conditions across the area and abundant waterhyacinth populations occurred west of Houma to the Atchafalaya Basin.

204. No evidence was found that *Sameodes* had become established at either Grand Lake in the Atchafalaya Basin or Pecan Island west of Lafayette. However, these releases were made only 2 or 3 months prior to the October survey, so it is not surprising that *Sameodes* was not detected.

205. *Sameodes* had become established on waterhyacinth in a large portion of southern Louisiana by the end of 1981. However, *Sameodes* had not dispersed to either the expansive Atchafalaya Basin or the vast marshlands west of Houma, and no evidence of *Sameodes* was found in central or northern Louisiana. *Sameodes* distribution will probably expand naturally to waterhyacinth populations throughout southern and central Louisiana, but efforts will be needed to establish the species in northern Louisiana. Waterhyacinth populations in this area are usually isolated and separated by large distances, which could preclude natural establishment of *Sameodes*.

206. *Sameodes* produced perceptible impacts on waterhyacinth populations at several sites in 1980 and 1981. The most readily observed impacts included significant brown-out areas in otherwise healthy waterhyacinth mats, and areas of open water or stunted waterhyacinths. Nearly all plants were damaged in areas of extremely dense *Sameodes* populations. However, there were no observed instances in which *Sameodes* greatly reduced the waterhyacinth population. Although it was too premature to predict the magnitude of future impacts on waterhyacinth in Louisiana, these observations suggested that *Sameodes* alone will not effect a major reduction of the waterhyacinth population. Considering its preference for the small, Stage I waterhyacinth morphotype, the major impact of *Sameodes* may be to limit the reproductive potential of infested plants. Severe damage by *Sameodes* larvae will destroy both the apical meristem and many lateral meristems. This inhibits production of both daughter plants and inflorescences. However, damage by *Sameodes* is minimal when waterhyacinth plants convert from the Stage I morphotype to the Stage III morphotype. Thus, other biocontrol agents (e.g. *Neochetina*) are needed to impact the Stage III plants.

207. The apparent inability of *Sameodes* to overwinter in large numbers in Louisiana may limit its effectiveness as a biocontrol agent. Should the current population development pattern persist, *Sameodes* will be relatively ineffective in many areas. Maximum impacts will occur only if large numbers of individuals overwinter and are available to allow the species to develop to

significant population levels during early spring, when most waterhyacinth populations consist of Stage I plants. This would not only provide maximum impacts on waterhyacinth when it is most susceptible to *Sameodes* damage, but would also provide potential for the development of extremely large populations of *Sameodes* later in the growing season.

Conclusions

208. Conclusions of the study were:

- a. *Sameodes* became established on waterhyacinth in southern Louisiana in 1979 and successfully overwintered.
- b. *Sameodes* dispersed rapidly during 1980, and became distributed in a 1230-km² area, including all or portions of five parishes.
- c. *Sameodes* distribution expanded during 1981 to include a 2883-km² area, encompassing all or portions of nine parishes in southern Louisiana.
- d. Although population development was sufficient to impact waterhyacinth in several areas in 1981, *Sameodes* did not significantly reduce the waterhyacinth population in any area.
- e. Since the *Sameodes* population was still in the dispersal and development phases, the magnitude of its effects on waterhyacinth could not be predicted.

PART VI: DISCUSSION

209. This section of the report synthesizes information obtained on waterhyacinth and the evaluated biocontrol agents from various studies described in Parts IV and V.

Waterhyacinth

Seasonal waterhyacinth population dynamics

210. Waterhyacinth populations consist of bulbous-petioled (Stage I) plants at the beginning of the growing season. These plants typically represent vegetative regrowth from plants surviving the winter season, but initial plants in spring sometimes result from seed germination. Plant density is initially low, but the abundance of available light and space stimulates daughter plant production. Daughter plant production is maximal by April, and plant density peaks during early May. When the entire water surface has been covered by Stage I plants, reduced light penetrating the canopy stimulates reduced daughter plant production and triggers a transformation of Stage I plants to long-petioled, taller Stage III plants. An intermediate morphotype (Stage II), in which the plants have both types of petioles and flowering is maximal, persists for a short time between the Stage I and Stage III morphotypes. As plants convert to the Stage III morphotype, intraspecific competition and reduced daughter plant production combine to decrease plant density. Plant height and biomass production increase until late summer (September-October). Plant density and daughter plant production are normally at their lowest levels at this time. Freezing temperatures at the onset of winter result in progressive destruction of waterhyacinth leaves and petioles. As plant buoyancy decreases, the plants float lower in the water. This occurs to a greater degree in Stage I plants than in Stage III plants. Most waterhyacinth tissues above the water surface are dead by spring, but the rhizome is usually not totally destroyed. These rhizomes produce the initial plants of the following growing season.

External stress factors

211. The pattern of waterhyacinth population development described above

is repeated annually when not influenced by external stress factors. However, the pattern is routinely disrupted in many areas of Louisiana by herbicide applications. Surviving plants are stimulated to increase daughter plant production when Stage III plants are treated with herbicides. The resulting waterhyacinth population is initially composed of Stage I plants, which then undergo the same progression of development to Stage III plants as described in paragraph 210. Interruptions in the normal pattern of population development (e.g. decreased biomass and height and increased density and daughter plant production) are indicators of external stress on a plant population. These changes may occur rapidly (e.g. herbicide applications) or slowly (e.g. biocontrol agents), and may persist for varying periods.

Population changes from 1974 to 1981

212. The LDWF conducts annual ground and aerial reconnaissance surveys of the waterhyacinth population in Louisiana during October, when the plant population is maximal. Survey results are synthesized to produce an estimate of total acreage of waterhyacinth. Annual estimates of the waterhyacinth population in Louisiana from 1974 through 1981 (Figure 2) revealed that the waterhyacinth population averaged 1.2 million acres during 1974-1978, declined slightly to 850,000 acres in 1979, and sharply decreased to approximately 320,000 acres in 1980 and 1981.

Factors influencing the decline in waterhyacinth populations

213. The significant reduction in the waterhyacinth population in Louisiana in 1980 and 1981 could not be explained as a normal population cycle. A similar decline in the waterhyacinth population in Louisiana had not previously been reported. Waterhyacinth was absent from many areas in 1980 and 1981 that previously had massive populations annually for 20 or more years. Three factors apparently contributed to the observed decline, including: improved herbicide spray program, the drought of 1980, and biocontrol agents.

214. Improved herbicide spray program. Modifications in herbicide spray programs resulted in greater efficiency of application and improved control of waterhyacinth. Better application systems, more intensive monitoring of waterhyacinth population development in high-use areas, and better trained applicators prevented massive population development in many areas. The use of helicopters enabled herbicide applications in many backwater areas that

could not be treated by conventional methods. However, the total acreage treated with herbicides did not increase significantly during 1980 and 1981, and was much less than the observed reduction in the waterhyacinth population. Thus, improved herbicide spray programs alone could not account for the significant reduction in the plant population.

215. Drought of 1980. Abnormally low precipitation during the first three quarters of 1980 resulted in dewatering of many shallow, backwater areas for most of the growing season. Waterhyacinth populations in these areas were either temporarily eliminated or greatly reduced. This was especially true in the large, backwater areas of the Atchafalaya Basin. Waterhyacinth populations are often flushed from backwater areas during high-flow periods into high-use canals, rivers, and lakes, thus necessitating herbicide applications. The failure of this to occur to a significant degree in 1980 contributed to the reduced acreage of waterhyacinth. However, normal precipitation during the winter of 1980-1981 resulted in rewatering of these areas. Conditions were ideal for rapid redevelopment of waterhyacinth populations from remaining plants and seed germination, and normal populations of waterhyacinth should have been present in these areas by October 1981. In addition, it was expected that waterhyacinth populations in areas not dewatered in 1980 would expand rapidly in 1981. However, the LDWF survey in October 1981 revealed no significant increase in the waterhyacinth population. This suggested that other factors were also significantly limiting the waterhyacinth population.

216. Biocontrol agents. There was abundant evidence that biocontrol agents, principally *Neochetina*, contributed significantly to the observed decline in the waterhyacinth population in Louisiana in 1980 and 1981. This evidence is presented in the following paragraphs.

Biocontrol Agents

Neochetina

217. Population dynamics. Relatively low numbers of both adult and larval *Neochetina* occur at the onset of the growing season. Both life stages are capable of overwintering, but significant mortality occurs during the winter. Population densities of both life forms normally increase to maximum levels by early fall. Two patterns of population development were observed in Louisiana.

Most commonly, the *Neochetina* population developed in a synchronous fashion (Figure 31), in which larval populations were relatively high when adult populations were relatively low. As the relatively larger larval populations completed development, adult populations increased. This pattern of population development was observed at the Lake Theriot, Amelia, and Centerville study areas. An asynchronous pattern (Figure 30) of population development occurred at Cypress Canal, in which peaks in larval and adult populations occurred simultaneously. *Neochetina* populations appeared to be increasing at all study sites, but increases were most pronounced at Amelia and Lake Theriot.

218. Historical development of the *Neochetina* population in Louisiana.

Neochetina was initially released in Louisiana in 1974 by the LDWF. Concerted release efforts by the LDWF and LMN in 1976 resulted in establishment of *Neochetina* on waterhyacinth throughout southern Louisiana. Population development was initially slow, due to the natural dispersal of the species to waterhyacinth populations in areas adjacent to release sites and because no more than three generations were possible in one year. *Neochetina* had become established in most areas by 1978, and populations in many areas had developed to sufficient levels to produce noticeable impacts on the plant populations. Relatively mild winters in 1978 and 1979 were conducive for rapid expansion of the *Neochetina* population, and by late summer 1980, adult populations in some areas reached such proportions that a "swarming" phenomenon was observed. Large numbers of *Neochetina* were removed from buildings at Pierre Part (Terrebonne Parish). These insects, which are capable of flight, were apparently attracted to the area by mercury-vapor lights near the buildings. *Neochetina* occurred at sufficient levels in 1980 to produce significant reductions in waterhyacinth populations in many areas.

219. Effects on waterhyacinth. *Neochetina* was the major factor producing the rapid, 90-percent reduction of the waterhyacinth population at Amelia in 1980. This decline was sufficient to preclude efforts to determine an optimum field-application rate for *Cerecospora*. The population dynamics of waterhyacinth and *Neochetina* suggested that the population density of *Neochetina* early in the growing season exceeded the threshold required to prevent the normal pattern of waterhyacinth development of the very small Stage I plants present on the site. The weevils eliminated most photosynthetic surfaces of leaves and interrupted normal translocation of water and nutrients.

The resulting decrease in biomass production was sufficient to inhibit normal conversion of the Stage I plants to the Stage III morphotype. Increased plant stress, produced as the insect population increased during the summer, resulted in death of most plants. Since this was the same year when the waterhyacinth population in Louisiana declined from 850,000 acres to 305,000 acres (Figure 2), this pattern was probably repeated in many areas. Although impacts of this magnitude were not observed elsewhere, waterhyacinth biomass, plant density, and/or percent cover declined in all other study areas in 1980 and 1981. The failure of *Neochetina* to produce similar effects on waterhyacinth in these areas was attributed to the relatively low populations of *Neochetina* during the early spring. Weevil population development in these areas apparently did not reach the threshold required to prevent the waterhyacinths from converting to the Stage III morphotype. Thus, late-season impacts of *Neochetina* were less. Nevertheless, *Neochetina* significantly impacted waterhyacinth on all study areas, which supports the conclusion that *Neochetina* was a major factor in the reduction of the waterhyacinth population in 1980 and 1981.

220. Threshold for impacts by *Neochetina*. The period covered by this report was too limited to allow definitive conclusions regarding the threshold population of *Neochetina* required to significantly reduce waterhyacinth populations. However, the significant reduction in percent cover, biomass, and density of waterhyacinth at the Amelia site during 1980 allowed a tentative assignment of threshold values. The number of weevils per plant at Amelia was not significantly higher than those at other study sites in which lesser reductions in biomass occurred. However, when insect density was portrayed as number of combined (adults and larvae) individuals per kilogram of waterhyacinth tissue adjusted by plant height, values for insect densities at Amelia were much higher than for other sites. A tentative value of 1.0 individuals per kilogram of plant tissue adjusted by plant height was established as the threshold for impacts of *Neochetina* on waterhyacinth. Longer periods of monitoring of other study sites will be necessary to determine whether or not this is the actual threshold value or whether the value should be somewhat lower. It is highly probable that the threshold value must be sustained for several generations to achieve significant reduction in the plant population. Although no data are available on the insect population density at Amelia

prior to 1980, the population present in May 1980 suggested that dense weevil populations were present on the site in 1979. Thus, reduction in the plant population in 1980 probably represented the latter stages of a sustained insect population that effected a significant reduction in the waterhyacinth population.

Cercospora

221. Although *Cercospora* was reisolated from waterhyacinth at all three sites where it was applied, significant population development occurred only at Lake Theriot. There was evidence in October 1981 that *Cercospora*, in conjunction with an expanding *Neochetina* population, was significantly impacting waterhyacinth at the site. The primary impact of *Cercospora* appeared to be acceleration of senescence of waterhyacinth leaves and petioles. As the period of active photosynthesis by individual leaves was reduced, total biomass production of waterhyacinth decreased.

222. The significant population development of *Cercospora* at Lake Theriot confirmed that: (a) viable propagules in the original formulation were infectious on waterhyacinth, (b) an application rate of 5.0×10^5 CFU/m² of *Cercospora* was sufficient to achieve significant infection; and (c) the original formulation could be mass applied by aerial application equipment. The original formulation applied at the Amelia site did not result in significant population development. This was attributed to the rapid decline of the waterhyacinth population caused by *Neochetina* damage. Feeding activity by *Neochetina* adults on the small plants destroyed the epidermis of most leaves, and the normal infection process of *Cercospora* was disrupted. *Cercospora* entry into waterhyacinth occurs through stomata, and removal of the leaf epidermis by *Neochetina* destroyed most stomata. Significant desiccation of subepidermal waterhyacinth tissues also resulted, which created unfavorable conditions for proliferation of *Cercospora*. Thus, potential for *Cercospora* infection and population development was greatly reduced.

223. The failure of *Cercospora* to become established on waterhyacinth at Centerville in 1981 was attributed to low infectivity of viable propagules in the modified formulation. The low infectivity could have resulted from unfavorable microenvironmental conditions that limited the growth potential of initial hyphae around the smaller particles of the modified formulation. However, the low infectivity of *Cercospora* in the modified formulation probably

resulted from low virulence of the fungus. This could have resulted from either loss of virulence in the stock *Cercospora* cultures used for production of the formulation or from some modification in formulation processing. The failure to achieve infection on greenhouse plants supported the hypothesis that loss of virulence was the major factor for failure of *Cercospora* to impact waterhyacinths at Centerville.

224. This study was of too limited duration to define the potential impacts of *Cercospora* on waterhyacinth in Louisiana. Nevertheless, *Cercospora* did become established on waterhyacinth at Lake Theriot, and the fungus was beginning to produce significant impacts on the plant population. However, there was no evidence that *Cercospora* was dispersing to other nearby waterhyacinth populations. This suggested that natural dispersal of *Cercospora* occurs at a very slow rate, probably because it is not an aggressive pathogen. Although *Cercospora* probably will not provide significant levels of waterhyacinth control in Louisiana when used alone, its potential for impacting waterhyacinth is sufficient to warrant its further distribution in Louisiana. Its greatest potential as a biocontrol agent will be in backwater areas where waterhyacinth populations proliferate.

Nameodes

225. *Nameodes* had become established on waterhyacinth in a large portion of southern Louisiana by October 1981 and had reached sufficient population levels to produce visible impacts on waterhyacinth population in some areas. Its potential as a biological agent for control of waterhyacinth in Louisiana is not yet known. Although significant population development was observed during late summer and fall, impacts on the waterhyacinth population will occur only if *Nameodes* can overwinter in sufficient numbers to allow rapid population development during the early spring months when most waterhyacinth populations consist predominantly of the Stage I morphotype. If this does not occur, impacts of *Nameodes* will be limited to those areas in which waterhyacinth populations are routinely treated with herbicides. As plants surviving the herbicide applications begin to regrow and multiply, suitable plants for *Nameodes* development will be present. Under these conditions, the major effect of *Nameodes* will be to limit the rate of waterhyacinth regrowth. This could be of importance in overall efforts to control waterhyacinth in Louisiana by reducing the number of required herbicide applications in high-use areas.

226. Continued expansion of the range of *Sameodes* in Louisiana is expected during the next 2 to 3 years. Since *Sameodes* adults are highly mobile and waterhyacinth populations in southern and central Louisiana are contiguous, the range of *Sameodes* should expand throughout the entire area without additional releases. Should this fail to occur, *Sameodes* will need to be released in the upper portion of the Atchafalaya Basin, in marshes of western Louisiana, and in central Louisiana. Additional releases will probably be required to establish *Sameodes* on isolated waterhyacinth populations in northern Louisiana.

Combinations of biocontrol agents

227. The effectiveness of *Neochetina* as a waterhyacinth biocontrol agent in Louisiana has been demonstrated. The ability of *Cercospora* to significantly reduce waterhyacinth populations has not been conclusively demonstrated, although it appeared to contribute to a reduction in waterhyacinth biomass at Lake Theriot. However, *Cercospora* is not widely distributed on waterhyacinth in Louisiana. *Sameodes* has become established in a large portion of southern Louisiana, but its level of impact on waterhyacinth remains to be determined. Despite the fact that evaluation of combinations of biocontrol agents could not be conducted during the LSOMT, all three species have potential for stressing waterhyacinth populations. Since waterhyacinth has tremendous growth potential in Louisiana, all three species should be utilized to place maximum stress on the plant population. *Neochetina* and *Cercospora* can be effectively utilized in combination to reduce biomass production and percent cover in areas where the waterhyacinth population consists predominantly of Stage III plants. Effects of *Sameodes* will largely be restricted to areas in which the waterhyacinth population consists predominantly of Stage I plants. In such cases, impacts of *Sameodes* may be enhanced by the presence of moderate to dense populations of *Neochetina*. *Sameodes* and *Neochetina* in combination will not be effective in areas where the waterhyacinth population consists predominantly of Stage III plants because *Sameodes* produces little direct impact on this morphotype. *Cercospora* and *Sameodes* are not compatible in combination because *Cercospora* produces relatively few impacts on Stage I plants that are preferred by *Sameodes*, while *Sameodes* impacts are minimal on Stage III plants that are most susceptible to *Cercospora* damage.

Prospects for biocontrol
of waterhyacinth in Louisiana

228. Despite the fact that *Neochetina* was the principal factor responsible for the reduction in the waterhyacinth population that occurred during 1979 to 1981, it is unlikely that biocontrol agents will ultimately reduce the waterhyacinth population to a nonproblem level. This is due to the tremendous growth potential of waterhyacinth and the fact that population levels of insects and plant pathogens are directly dependent on the population levels of waterhyacinth. As biocontrol agents reduce waterhyacinth populations, their populations will also decline. When this occurs, natural pressures on waterhyacinth will be reduced, thereby allowing the waterhyacinth population to rapidly redevelop. As the population of waterhyacinth increases, populations of insect and pathogen biocontrol agents will increase. However, the rate of population development of biocontrol agents will be slower than that of waterhyacinth. Thus, there will continue to be periods in which waterhyacinth populations occur at problem levels. The degree of long-term control of waterhyacinth afforded by biological agents will depend largely on the rate at which their populations increase following periods of waterhyacinth regrowth. A natural cycling of waterhyacinth and biocontrol agent populations will probably occur, but the magnitude of regrowth of the waterhyacinth population and the period between cycles cannot be predicted at this time.

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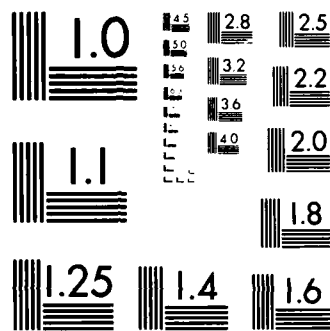
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PART VII: CONCLUSIONS

229. General conclusions of the LSOMT studies were:

- a. The waterhyacinth population in Louisiana declined by approximately 70 percent during 1979 to 1981 from an average of 1.25 million acres (1974-1978) to slightly more than 300,000 acres in 1980-1981.
- b. The observed decline in waterhyacinth in Louisiana was attributed primarily to effects produced by *Neochetina*, but improved herbicide application programs and drought conditions during the 1980 growing season also contributed to the observed decline.
- c. Waterhyacinth biomass and percent cover decreased in all LSOMT demonstration studies in association with increasing populations of *Neochetina*.
- d. *Cercospora* became established at Lake Theriot and appeared to contribute to the observed decline in the waterhyacinth population.
- e. *Cercospora* in the modified formulation applied at Centerville lacked sufficient virulence to infect the waterhyacinth population.
- f. *Sameodes* originally became established on waterhyacinth in the Cypress Canal area, dispersed rapidly, and its October 1981 distribution included a 2883-km² area in all or portions of nine parishes.
- g. Due to problems in controlling the distribution of biocontrol agent populations after their release and the failure of *Cercospora* to infect waterhyacinth at the Centerville site, it was not possible to document effects of the various combinations of biocontrol agents on waterhyacinth populations.
- h. Biological agents are expected to provide long-term control of waterhyacinth in many areas of Louisiana, but are not expected to reduce the state-wide waterhyacinth population to a non-problem level in all areas. A natural cycling of biocontrol agent and waterhyacinth populations is expected to occur.

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Table 1
Plot Means for Waterhyacinth Parameters Monitored During
the *Cercospora* Field Application Rate Study

Parameter	April 80	July 80	September 80
Percent cover	89.9	33.6	10.2
Plant density, #/m ²	116.7 (±8.65)*	74.8 (±8.78)	40.5 (±7.59)
Plant density--weighted, #/m ² * % cover	104.8	25.2	4.1
Biomass, kg/m ²	11.8 (±6.36)	6.1 (±1.38)	5.6 (±0.78)
Biomass--weighted, kg/m ² * % cover	10.6	2.1	0.6
Plant height, cm	8.0 (±0.62)	22.2 (±3.03)	24.6 (±4.84)
Daughter plants, #/m ²	31.3 (±6.04)	7.2 (±2.62)	6.5 (±3.79)

* Numbers in parentheses represent two standard errors of means.

Table 2
Plot Means of Pathogen Damage Per Leaf During the *Cercospora*
Field Application Rate Study

Treatment Rate*	April 1980	July 1980	September 1980
10 ⁴	3.26	2.92**	7.71†
10 ⁵	3.22	2.89	6.95
10 ⁶	3.15	3.06	7.35
C	3.42	3.60	— ††

Note: Means were calculated from three replicates of each treatment rate except where indicated.

* Treatment rates expressed as number of colony forming units per square metre.

** Based on two replicate plots.

† Based on one replicate plot.

†† Too few plants remained to allow sampling.

Table 3
Plot Means for Waterhyacinth Parameters Monitored
at the Lake Theriot Study Area

Parameter	May 1980	Jul 1980	Oct 1980	Apr 1981	Jul 1981	Sep 1981
Percent cover	100.0	100.0	100.0	100.0	100.0	100.0
Plant density, #/m ²	201.0 (±16.06)*	100.7 (±6.97)	53.7 (±5.80)	110.7 (±10.53)	110.7 (±9.12)	66.3 (±5.23)
Biomass, kg/m ²	19.5 (±1.36)	30.4 (±1.60)	19.2 (±1.64)	5.7 (±0.63)	12.8 (±1.27)	13.2 (±1.13)
Plant height, cm	31.2 (±1.94)	70.6 (±9.84)	77.1 (±3.27)	16.8 (±0.99)	39.2 (±2.34)	52.9 (±3.23)
Daughter plants, #/m ²	17.6 (±4.76)	0.8 (±0.71)	3.5 (±2.32)	32.8 (±8.14)	6.5 (±2.25)	2.1 (±0.92)

* Numbers in parentheses represent two standard errors of means.

Table 4
Mean *Arzama* Larvae and Mean Index Values for *Orthogalumna*
at the Lake Theriot Study Area

	May 80	Jul 80	Oct 80	Apr 81	Jul 81	Sep 81
<i>Arzama</i> larvae*	2.6	0.4	0.9	0.0	0.0	0.5
<i>Orthogalumna</i> **	0.1	0.7	0.6	0.0	0.0	0.0

* Larvae/m².

** Index of *Orthogalumna* tunnels/leaf.

Table 5
Plot Means for Waterhyacinth Parameters Monitored
at the Centerville Study Area

<u>Parameter</u>	<u>Aug 1980</u>	<u>Apr 1981</u>	<u>Jul 1981</u>	<u>Sep 1981</u>
Percent cover	100.0	100.0	100.0	100.0
Plant density, #/m ²	54.9 (±6.49)*	91.9 (±9.53)	101.6 (±15.48)	52.5 (±9.10)
Biomass, kg/m ²	21.5 (±1.46)	4.7 (±0.44)	14.7 (±1.25)	17.3 (±1.21)
Plant height, cm	70.6 (±3.50)	18.6 (±1.87)	54.0 (±7.28)	70.2 (±6.31)
Daughter plants, #/m ²	4.7 (±1.88)	43.7 (±4.49)	6.0 (±2.19)	3.1 (±1.79)

* Numbers in parentheses represent two standard errors of means.

Table 6
Mean *Arzama* Larvae and Mean Index Values For *Orthogalumna*
at the Centerville Study Area

	<u>Aug 80</u>	<u>Apr 81</u>	<u>Jul 81</u>	<u>Sep 81</u>
<i>Arzama</i> larvae*	0.0	0.0	0.2	0.0
<i>Orthogalumna</i> tunnels**	0.0	0.0	0.0	0.0

* Larvae/m².

** Index of *Orthogalumna* tunnels/leaf.

Table 7
Plot Means for Waterhyacinth Parameters Monitored
at the Cypress Canal Study Area

Parameter	May 1980	Jul 1980	Oct 1980	Apr 1981	Jul 1981	Sep 1981
Percent cover	100.0	100.0	100.0	80.0	75.0	60.0
Plant density, #/m ²	112.5 (±12.89)*	43.5 (±4.25)	28.5 (±2.79)	148.9 (±12.37)	80.7 (±5.57)	25.2 (±2.87)
Plant density-- weighted, #/m ² * % cover	112.5	43.5	28.5	119.1	60.5	15.1
Plant biomass, kg/m ²	7.8 (±0.89)	7.8 (±1.16)	10.9 (±1.44)	8.5 (±0.69)	8.1 (±0.86)	10.9 (±1.33)
Plant biomass-- weighted, kg/m ² * % cover	7.8	7.8	10.9	6.8	6.1	6.6
Plant height, cm	23.1 (±1.30)	51.1 (±4.80)	56.3 (±2.75)	21.1 (±1.11)	42.3 (±2.16)	65.3 (±5.62)
Daughter plants, #/m ²	13.2 (±4.94)	13.7 (±4.20)	2.3 (±1.58)	60.7 (±11.94)	11.6 (±5.26)	13.7 (±3.91)

* Numbers in parentheses represent two standard errors of means.

Table 8
Mean *Arzama* Larvae and Mean Index Values For *Orthogalumna*
at the Cypress Canal Study Area

	May 80	Jul 80	Oct 80	Apr 81	Jul 81	Sep 81
<i>Arzama</i> larvae*	0.8	0.3	0.0	0.0	0.0	0.0
<i>Orthogalumna</i> tunnels**	0.0	0.0	0.6	0.0	0.2	0.6

* Number of larvae/m².

** Index of *Orthogalumna* tunnels/leaf.

Table 9

Light Trap Collections of *Sameodes* at Edgard, La.

<u>Date</u>	<u>Adults Captured</u>
1980	
30 May	1
2 Jun	1
2 Sep	1
5 Sep	1
12 Oct	1
1981	
20 Jul	1
25 Jul	1
23 Aug	3
24 Aug	1
6 Sep	1
28 Sep	1
30 Sep	2
10 Oct	3
13 Oct	1
16 Oct	2

Table 10

Locations at Which *Sameodes* Occurred in Louisiana in 1981

St. Charles Parish

Cypress Canal (two locations)

Louisiana Department of Wildlife and Fisheries Game Management Area
Headquarters (Lake Salvador)

Umbrella Canal west of Lake Catawatchee

Sellers Canal at US Highway 90

Jefferson Parish

Canal paralleling US Highway 90 (two locations)

Lafourche Parish

Canal paralleling US Highway 90, 6.6 km east of junction with Louisiana
Highway 316

Terrbonne Parish

Canal at Louisiana Highway 24 and US Highway 90 intersection

St. John the Baptist Parish

Edgard (five adults collected in light trap)

Table 11

Locations at Which *Sameodes* Occurred in Louisiana in 1981

St. John the Baptist Parish

Canals at intersection of Interstate-55 and Interstate-10 (two sites)
 Canal paralleling east side of Interstate-10, 1.6 km south of weighing station
 Canal paralleling west side of Interstate-10 at weighing station
 Edgard (light trapping by Vernon Brou)

Jefferson Parish

Canal paralleling US Highway 90, 16.1 km west of Westwego

Lafourche Parish

Bayou Des Allemands at US Highway 90 intersection

Terrebonne Parish

Canal paralleling US Highway 90, 0.8 km east of Houma
 Bayou Terrebonne at E. Main St. (Houma)
 Canal paralleling Louisiana Highway 315 at Henry Clay St. intersection near Houma
 Canal paralleling Louisiana Highway 315 at public boat launch in Theriot
 Bayou Black, 4.9 km west of Houma
 Bayou Black, 8.0 km west of Houma at pumping station

St. Charles Parish

Cypress Canal (Lake Salvador)
 Sellers Canal at US Highway 90
 Canal paralleling US Highway 90, 1.6 km west of Sellers Canal
 Canal paralleling US Highway 90 at Paradis
 Canal paralleling US Highway 90, 3.2 km west of Paradis
 Canal paralleling US Highway 61 at Norco (two sites)

St. James Parish

Canal paralleling US Highway 61, 1.6 km west of Gramercy

Ascension Parish

Canal intersecting Interstate-10 south of mile marker 188
 Canal paralleling east side of US Highway 61, 3.2 km north of Interstate-10 intersection
 Canal intersection US Highway 61, 1.6 km south of Sorrento

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